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कृपया किताबों को स्वच्छ रखने में सहायता करें।

LEWIS RALPH
1864-1945

IARI

G. W. KEITT AND F. V. RANDI

Lewis Ralph Jones, Professor Emeritus of Plant Pathology of the University of Wisconsin, died at Orlando, Florida, on April 1, 1945, in his eighty-first year. In his passing the University of Wisconsin has lost one of her greatest scholars, the American Phytopathological Society one of its founders and leading spirits, and biological science one of its most constructive leaders. His many students throughout the world have lost a beloved teacher and trusted friend.

His forebears were vigorous and able pioneers in the settlement of this country. His father, David Jones, born of English parents in 1828 at the border of England and Wales, came to America with his family at the age of fourteen. After three years at Utica, New York, the family settled on a farm near Kenosha, Wisconsin. At the age of twenty, David bought a tract of government land in Metomen township of Fond du Lac County, near the site on which the village of Brandon later was established. Here he built his house and lived a long and useful life as a successful farmer and a leader in the community. He raised Guernsey cattle, and his oats won a bronze medal at the St. Louis Exposition. In 1856, he married Lucy Jane Knapp, who was descended from a long line of New England and Pennsylvania Dutch ancestors, some of whom became pioneers in western Vermont soon after the Revolutionary War. Lucy Jane, daughter of Alva and Mary Cuthbert Knapp, was born at Starksboro, Vermont, May 27, 1837. In 1850, her parents moved to Metomen township, and later to Brandon. Before her marriage she was teacher of the local district school. She was a woman of much wisdom and charm and a discriminating reader with broad interests. From girlhood in one of the most picturesque of the Green Mountain valleys she brought a love of nature, and especially of plant life, that she early imparted to her children. She was devoted to her family and to the cultural and religious life of the community. Hers was the dominant influence in making it possible for her son, Ralph, who early showed the bent of a scholar, to prepare himself for a liberal education.

Lewis Ralph, the third of six children of David and Lucy Knapp Jones, was born on December 5, 1864. In their busy home of high ideals and sterling values were laid the foundations of the character that was to guide him so well. His early education was at Brandon. From 1883 to 1886 he attended Ripon College, where he became attracted to biological science under the stimulating influence and helpful counsel of C. Dwight Marsh, Professor of Chemistry and Biology. For more advanced studies he went

¹ Grateful acknowledgments are made to colleagues in the Department of Plant Pathology of the University of Wisconsin and to many other former students and friends of Professor Jones for their assistance in the preparation of this sketch of his life and works.

in 1886 to the University of Michigan. His undergraduate education was oriented towards medicine, but in his senior year he decided to go into plant pathology instead. In making this decision, he was much influenced by Dr. Volney M. Spalding, Professor of Botany, and Erwin F. Smith, then a mature and exceptionally brilliant graduate student who was already well started in his distinguished career in the United States Department of Agriculture. In his last semester as an undergraduate he was privileged to attend Smith's doctoral examination. In his own words: "This examination covered Smith's early work on peach yellows. The glimpses thus given me of the significance of and opportunity for research in the field of plant pathology were most inspiring. . . . In conference with my wise and revered counselor, Professor Volney M. Spalding, the decision to turn to plant pathology was promptly reached and never since regretted."

The arresting fact that the University of Michigan should at this time have launched upon their careers two young men who were to become outstanding leaders of their generation in both plant pathology and the broader field of biology and agriculture suggests some consideration of background events. Great developments in American education and research were in the making, the University of Michigan was becoming one of the foremost centers of learning in this country, and the pioneer homes of the Middle West were producing young men with the character, ideals, intellect, vigorous initiative, and sympathetic understanding of needs that enabled them to meet the great calls for service that so rapidly developed.

Plant pathology in America was in its infancy at a time when a rapidly expanding agriculture was presenting plant disease problems of great urgency. The foundations of this new science had been laid in Europe by such pioneers as Prévost, the Tulasnes, de Bary, and Kühn, and similar advances had been made in the field of infectious diseases of animals and man by Koch, Pasteur, Lister, and others. Farlow had studied under de Bary in the mid-seventies and brought the inspiration and methods of this great leader to Harvard, where he had begun training men, especially in the taxonomy and life-histories of parasitic fungi. Burrill, at the University of Illinois in 1878 to 1881, had made the classic discovery that bacteria incite the fire blight disease of apples and pears, and pioneer work on plant diseases was under way in many states. By 1885, Millardet and others in France had discovered Bordeaux mixture and demonstrated such dramatic success in its control of destructive plant diseases that great impetus had been given to the development of plant pathology and related fields of agricultural science. A section of mycology, devoted primarily to work on plant diseases, had been established in the U. S. Department of Agriculture in 1886, and in 1887 the Hatch Act had made possible an agricultural experiment station in each state.

In the mid-eighties, the University of Michigan was rapidly developing in scientific strength and leadership. Dr. Victor C. Vaughan was beginning the program in medical bacteriology that he and Dr. Frederick V. Novy

continued with such success. Professor Volney M. Spalding sensed the great needs and opportunities in the new field of plant pathology and encouraged his students to work on plant diseases. Once they had the opportunity to engage in such studies and envision the needs for service and the opportunities for scientific contribution in this field, it was inevitable that men with the splendid natural aptitudes and fine spirit of Smith and Jones should enter it with all their energies and enthusiasm.

Jones' first teaching experience came between his junior and senior years, when he served as Instructor in Natural Science at Mt. Morris Academy, Mt. Morris, Illinois, for the year of 1887 and two terms of 1888. The success of this early teaching is attested by the life-long friendship of members of his classes.

Upon completing his course and receiving the Ph.B. degree in 1889, he was called to the University of Vermont, where he served as Botanist of the Agricultural Experiment Station, 1889-1910, Instructor in Natural History, 1889-91, Assistant Professor of Natural History, 1891-92, Associate Professor of Natural History, 1892-93, and Professor of Botany, 1893-1910.

On June 24, 1890, he married May I. Bennett, a classmate at Ripon College. She shared generously in his life's effort, making many personal sacrifices to help him meet the urgent pressure of work during his early and middle years and keeping their hospitable home always open to his students and other friends. She passed away peacefully in her sleep on September 22, 1926.

His twenty years at the University of Vermont, interspersed with travel and special studies at the University of Michigan, in the United States Department of Agriculture, and in Europe, marked the first stage of Professor Jones' career and established him as a leader in American botany and plant pathology. In this early formative period in the development of the state agricultural experiment stations, the United States Department of Agriculture, and the state colleges and universities, his contribution was rich and varied. Most men are fortunate if they can render distinguished service as teacher, investigator, or administrator and builder in education and research. Professor Jones made distinguished contributions in all of these fields.

He became one of the most effective and beloved teachers in the history of the University of Vermont. He was held in the highest affection by his students and so great was his esteem by the student body that many who were concentrating in unrelated fields took work with him to experience the influence of his tutelage and personality. Some of these were so attracted that they continued in his field for their life work. During his last few years at Vermont over a score of students went out from his laboratories into professional work in plant science.

He was highly productive in research and in its practical applications, developing a national leadership in studies of the nature and control of plant diseases. His first important contribution was his pioneering in the

adaptation of Bordeaux mixture to use in America. Beginning with experiments in the summer of 1889, in which late blight of potato was successfully prevented, he rapidly developed methods for the practical control of that disease and of both apple and pear scab by this "marvelous new French fungicide." Examination of the appended bibliography will give some idea of the wide scope of the work that followed. The practical needs of agriculture and forestry were given all possible consideration. Duties that in other hands might have been routine were made to yield high educational values, and contacts with the farmers were made to contribute both to the solution of their problems and enlistment of their interest and cooperation. Notwithstanding the multiplicity of duties, certain problems were selected for intensive researches that resulted in contributions of the highest quality. Notable among these were his investigations of potato diseases, bacterial soft rot of vegetables, and sap flow in sugar maple.

The work on potato diseases included studies at the University of Michigan and in Europe, where from April to September of 1904 he visited leading laboratories in England, France, Belgium, Holland, and Germany and became acquainted with many of the foremost plant scientists and plant culturists of those countries. Commissioned by the United States Department of Agriculture to make collections of potato varieties that breeders of this country might use in the endeavor to develop stocks resistant to disease and superior in other qualities, he brought back over 90 varieties. These were studied at Vermont in association with Professor William Stuart, and turned over to Professor Stuart for further studies when the latter became leader of the potato breeding program of the United States Department of Agriculture. The classical paper on late blight of potato published in 1912 by Jones, Giddings, and Lutman as Bulletin 245 of the Bureau of Plant Industry remains the most comprehensive and authoritative publication on this important disease.

Another notable experience during the Vermont years was the six months spent in 1899 in Erwin F. Smith's laboratory in Washington, when at Smith's invitation he took his problem on bacterial soft rot of vegetables there for special studies. This was a very fortunate and significant experience for all concerned. It enabled him to renew his associations with Smith and brought him into close contacts with a group of outstanding leaders in the development of the Bureau of Plant Industry. Community of interest drew them together in informal seminars that met frequently in one or another laboratory. These were supplemented by lunching together and by "Dutch" dinners at Harvey's famous seafood restaurant. Besides Smith and Jones, regular attendants included A. F. Woods, B. T. Galloway, M. B. Waite, Mark A. Carleton, and Theodore Holm. These informal seminars later developed into the Botanical Seminar of the Bureau of Plant Industry and finally into the Botanical Society of Washington. In recalling these early experiences, Dr. Woods recently said: "The discussions were chiefly along bacteriological and physiological lines, and Jones was one of

the leading spirits. He was looked upon by the Department as a prize visitor. He brought to the group the university point of view. Though he came for further bacteriological training and experience as well as research, he gave fully as much as he got. We were proud to have him with us." Out of such relations grew mutual understanding and confidence, and the way was paved for life-long associations of great advantage to all concerned.

A further outcome of the investigations on bacterial soft rot of vegetables was the publication of Prof. Jones' classical papers describing *Bacillus carotovorus* and showing the mechanism of its incitation of rotting by production of the enzyme, pectinase, which dissolves the middle lamellae of the cell walls of the host plant.

In 1901 and 1904 periods of graduate study were spent at the University of Michigan, from which he received the Ph.D. degree in 1904.

Professor Jones' influence at Vermont extended far beyond the boundaries of his special field. He was a trusted counselor, both of students and colleagues, and a leading spirit in the life of the University. He was the leading organizer of the Vermont Botanical Club and the initiator and a President of the Vermont Forestry Association. The L. R. Jones State Forest of Vermont was named in recognition of his services to forestry. He was a leader in the movement that established training in home economics at Vermont, and was highly instrumental in starting teacher training work there. It is a tribute to his accomplishments as a teacher that he was asked to serve as the first Dean of the Department of Teaching. This assignment was carried in addition to his other work.

In 1909 Professor Jones was called to the University of Wisconsin to found its Department of Plant Pathology. Difficult as it was to leave the friends and associations in Vermont, the decision was made easy, to use his own words, . . . "since we could by returning to Wisconsin personally renew our early home associations and professionally meet the increasingly evident responsibility of opening thus, in a larger university particularly strong in botanical traditions and associations, opportunities for the training . . . of younger men and women beyond those which had been possible to us of the older generation." To his new task, which he took up on February 1, 1910, he brought the rare talents and devotion that were to win him recognition, not only as the dean of the plant pathologists of his generation, but also as one of the leaders of his time in the broad field of biology and agriculture.

He combined the highest ideals of scholarship with a first-hand understanding of the practical needs of agriculture and a vital interest in the applications of science to the service of mankind. He was rarely gifted in understanding and inspiring both his students and his colleagues, and gave of himself without reservation. He had the vision and the ability to integrate his new department closely with related departments of the University and with the U. S. Department of Agriculture, always fostering mutual

assistance and cooperation. From the beginning it was his policy, insofar as possible, to send his students for basic training to the related departments concerned, developing in plant pathology only research and such special courses as were deemed essential to supplement the basic training. Because of the close natural relationships of the two fields and the strength and cordial cooperation of the Department of Botany, work in plant pathology and botany was especially closely integrated. Much of the strength of the development in plant pathology was made possible by the splendid cooperation of Dr. R. A. Harper and his colleagues in the Department of Botany from the outset and by their successors throughout the years.

Professor Jones conceived of research as the central activity about which to build his department. Only by competent research on the basic problems of plant disease development and control could the practical needs of agriculture be met. Only upon the results of such research could adequate instruction be based. He knew that students learn best by doing, and early recognized the great advantages of closely integrating postgraduate teaching with the research program of the department. Never desirous of ambitious buildings or unnecessarily large appropriations, whose dangers he well recognized, he was ever earnest in urging provision of the facilities and funds necessary for the effective and economical development of the work of the department, and was exceptionally gifted in bringing together support from different sources for attack on common problems.

Students came to his laboratory from all over the world and have gone forth to most of our states and to many foreign lands with the benefits of his training and the stamp of his character. Though his objective was always quality rather than quantity, his department has trained 145 students as majors in plant pathology who have been awarded the Ph.D. degree by the University of Wisconsin. Professor Jones believed strongly in the value of the ancient custom of migration of students, and encouraged it, both in welcoming those from other institutions and countries and in advising his own students to broaden their horizons by studies elsewhere. Similarly, no opportunity was missed to encourage exchange of visits with qualified colleagues from other institutions.

Though the time that Professor Jones could devote to his individual researches in the years at Wisconsin was progressively limited by the multiplicity and weight of his other responsibilities, the quality of his work set a standard for all. In developing the research program, as in other lines of building the department, it was his method to select men carefully, assign their responsibilities, and then give them all possible freedom, support, and encouragement in their work. He realized that what he could accomplish by aiding in the development of men, programs, and institutions would greatly outweigh any personal research contribution he might hope to make. Furthermore, his greatest satisfaction was in assisting his students or colleagues to find themselves and in seeing their growth and accomplishments. He was scrupulously careful to see that all possible deserved credit went

to his students and associates. His contribution as director of the research work of his department and as a leader and counselor in the development of trends of research was of the highest importance. Notable examples, both of his personal research and his leadership in trends of research during the Wisconsin period, are his contributions to the study of disease resistance in plants, including development of disease-resistant varieties, and his work on the influence of environment on plant diseases. The late Dr. E. J. Butler,² distinguished Director of the Imperial Mycological Institute (Kew, Surrey, England), pointed to the high-light of Professor Jones' contribution as follows: "There are three phases in the history of plant pathology: First, the period of de Bary, in which the fungus held first place; second, the period in which the host received most attention; and finally, the present period in which disease is considered as an interaction of both under the conditioning influence of the environment. The leader in this is Jones."

The development of his department was but one of Professor Jones' great contributions during the Wisconsin phase of his career. His knowledge and experience, breadth of vision, wisdom, and rare constructive abilities were constantly in demand.

He contributed generously to the broader aspects of the development of the University. He was one of the founders and leading spirits of the Graduate Biological Division, a faculty group spontaneously organized to foster graduate work and related interests in the field of the biological sciences. He served on major committees of the University dealing with institutional policies, and his counsel was widely sought and highly regarded.

His contributions outside the University were varied and highly significant. One of special interest to readers of *Phytopathology* was his services to our Society, its members, and its journal. He was one of the founders and charter members of the American Phytopathological Society, its first president (1909), the first editor-in-chief of *Phytopathology* (1911-14), and a life-long inspiring leader in the work of the Society. He contributed largely to the rapid and sound development of the Society and to the vitality, practicality, and generous spirit of cooperation and service that have characterized it.

Professor Jones was also one of the leading spirits in the Botanical Society of America. He served as vice-president in 1910 and president in 1913. He was a member of the committee appointed to establish a journal for this society (1913) and served on the Editorial Committee of the *American Journal of Botany* (1914-16 and 1919-21).

At the end of the first world war, when many major responsibilities for scientific leadership shifted from Europe to America, Professor Jones' school of plant pathology at Wisconsin was fully established and he was ready and able to contribute largely to leadership in the broad field of biology and agriculture. He rendered valuable services through his rela-

² From remarks made at the banquet given in appreciation of Professor Jones by his former students at Ithaca, N. Y., August 19, 1926, as recorded by F. V. Rand.

tions in the National Academy of Sciences and the National Research Council, serving in the latter as vice-chairman of the Division of Biology and Agriculture from 1919 to 1921 and chairman in 1922. He was one of the founders of the Tropical Plant Research Foundation and served as president from 1924 to 1943. He was one of the organizers of the Boyce Thompson Institute for Plant Research and served on its Board for the rest of his life. In 1943 he was appointed by President Roosevelt to the Science Advisory Board.

There were many other special services or missions during these busy years. He found time while president of the Tropical Plant Research Foundation to go to Cuba as adviser on problems of sugar-cane culture. In 1926 he went to the Hawaiian Islands as consultant on problems of pineapple culture. He visited Puerto Rico in 1930 as adviser on agricultural developments. In 1930, he made an extensive tour of Europe and European Russia, visiting leading laboratories, conferring with colleagues, and renewing associations with his former students. This visit included participating in the Fifth International Botanical Congress (Cambridge, England), where he served as Chairman of the Section of Mycology and Plant Pathology. In 1931 and 1932, he traveled extensively with similar purposes in the Far East and the Islands of the Pacific, with special attention to Japan, Korea, China, the Philippine Islands, and Hawaii.

Professor Jones had the richly deserved good fortune to be greatly appreciated during his lifetime, receiving many honors at home and abroad. A list of his affiliations and honors is given on a later page.

An unusual honor that touched him very deeply was the reunion banquet given in his appreciation on the occasion of the presentation of his portrait to the University of Wisconsin by his former students, at the International Congress of Plant Sciences at Ithaca, N. Y., on August 19, 1926. High tributes for his many-sided contribution were paid by W. A. Orton, A. G. Johnson, I. E. Melhus, H. A. Edson, and G. W. Keitt, representing his former students, L. H. Bailey, R. A. Harper, and Erwin F. Smith, representing colleagues in the United States, and O. Appel and E. J. Butler, representing colleagues abroad. With the portrait were presented 22 volumes of the collected works of his students since 1910 to supplement the seven volumes that had been presented him when he left the University of Vermont. It is unfortunate that limitations of space prevent inclusion of an account of the substance of these tributes and of Professor Jones' response. It must suffice to include his closing remarks, which so well illustrate his ideal of service through science.

"Watching the trend in modern scientific developments as I have, I wish to warn you of one of the subtlest dangers facing you collectively as well as individually. I refer to the danger of professionalism. If you understand my meaning I think you will agree that with the present outlook, and especially in a young, rapidly developing country like ours, it is impossible to escape this danger altogether. You Vermont boys need only

to revision that great amateur botanist, Dr. Pringle, to understand what I mean by the spirit of amateurism as compared with professionalism—the devotion of one who is working for the love of science rather than for a living through science. And in Wisconsin we are happy in having another such in Dr. Davis. Unfortunately, the temptation to time-serving professionalism is stronger today than it was a generation ago, when, for example, in the entire membership of the Vermont Botanical Club I was perhaps the only one who was paid for studying plants.

“But, fortunately, on the other hand, the objectionable aspect of professionalism need not follow simply because one receives a living wage as a botanist. Quite as fortunately, the dangers are in no wise proportioned to the size of the salary. It all depends upon one’s attitude toward his work. I have no fear as to this if we can only continue in the spirit in which I am sure you all have begun—the spirit of devoted scientific service. I like to think of those in state or federal positions in plant pathology as holders of honorary fellowships, as it were, for public service to society through creative work in the field of the plant sciences. In this way the best of that characteristic of the amateur spirit may be adequately perpetuated in our professional group, let it grow to any size and sub-divide to any degree of specialization.”

The pressures of Professor Jones’ scientific responsibilities were not allowed to mar the graciousness of his life. He was the kindest and most considerate of men, ever available to his students and his colleagues for wise and sympathetic counsel. His students were always welcome in his home, and many of their happiest memories center about his hospitable fire-side. Always keenly interested and active in the cultural, civic, and religious life of his community, he was a member of the University Heights Poetry Club, the Madison Literary Club, the University Club, the Rotary Club, the Masonic Fraternity, and the Congregational Church.

On July 27, 1929, he married Anna M. Clark, one of his former students at Vermont, who survives him. A distinguished teacher in her own right, she shared fully in his interests, friendships, and hospitality; and after the condition of his health required restriction of activities her loving care and congenial companionship made his twilight years very happy ones.

In 1930, Professor Jones resigned the chairmanship of his department and asked to be put on part-time service in the University, in order to free additional time for travel and his outside scientific responsibilities. He retired from active service in 1935, but continued to carry important responsibilities in the National Academy of Sciences and as committee man, trustee, and counselor. The leisure of his later years permitted much of the travel that he and Mrs. Jones so much enjoyed. Always central in their travel plans was the continuation of his long-established custom of visiting colleagues and former students, ever radiating encouragement, inspiration, and good cheer. Except when interrupted by more extended travels, the winters were usually spent in Florida and the summers at Mrs. Jones’

ancestral home in Brookfield, Vermont, with a spring visit to Madison. In the winter of 1941, Professor Jones developed a heart condition that necessitated some restrictions of his activities. Nevertheless, he continued to enjoy very good general health and retained his keen interests and kind and happy associations to the last.

An event that touched him very deeply and is a lasting satisfaction to his many friends was the receipt upon his eightieth birthday of some 250 letters of affection and appreciation from former students and other friends from all over the world.

He passed away peacefully in his sleep during the night of March 31, 1945. He had been much interested for several days in formulating plans with Mrs. Jones for the coming months. Apparently he had no premonitions and suffered no pain.

Funeral services were held at the Congregational Church at Madison, of which he had been a member for 35 years, and interment was in the family lot at Brandon.

Though his personal researches were of the first rank, Professor Jones' greatest contribution was the impress he made on the lives of others and the constructive influence he exerted on institutions and programs of education and research in a critical formative phase of their development in North America. What he did was possible only because of what he was. His integrity and unswerving devotion to the highest ideals of science and of life, his clarity of vision and steadfastness of purpose, his deep and sympathetic understanding of human relations, and his unfailing kindness and untiring devotion to the service of others were a lasting inspiration to those who had associations with him. He will long be remembered for his leadership in science and his example in life.

DEGREES, HONORS, AND MEMBERSHIPS

University of Michigan, Ph.B. (1889), Ph.D. (1904), LL.D. (1935); University of Vermont, Sc.D. (1910); University of Cambridge, Sc.D. (1930); University of Wisconsin, Sc.D. (1936).

Editor-in-chief, *Phytopathology* (1911-14); editor, *American Journal of Botany* (1914-16, 1919-21), bacteriological terms in Webster's New International Dictionary of the English Language (1928); trustee, Boyce Thompson Institute for Plant Research (1924-45); president, Tropical Plant Research Foundation (1924-43); an honorary president, Third International Congress for Microbiology (New York, 1939).

Member, Academy of Natural Science of Philadelphia, American Association for the Advancement of Science (fellow; vice-president and chairman of Section O, 1924-25). American Philosophical Society, American Phytopathological Society (president, 1909). American Society of Naturalists, Association of Applied Biologists (England), Board of Park Commissioners, Burlington, Vt., Botanical Society of America (vice-president, 1910; president, 1913), Fifth International Botanical Congress (chairman.

Section of Mycology and Plant Pathology), Vermont Botanical Club, Vermont Forestry Association (president, 1908), National Academy of Sciences, National Research Council (vice-chairman, Division of Biology and Agriculture, 1919-21; chairman, 1922), New England Botanical Club, Phytopathological Society of Japan (honorary), Science Advisory Board (appointed by the President of the United States in 1934), Société de Pathologie végétale et Entomologie agricole de France (honorary), Verein für Angewandte Botanik (honorary), Wisconsin Academy of Sciences, Arts, and Letters, Gamma Alpha, Phi Kappa Phi, Phi Sigma, Sigma Xi (president Wisconsin chapter, 1914-15), Madison Literary Club, University Heights Poetry Club (Madison, Wis.), University Club (Madison, Wis.), Cosmos Club (Washington, D. C.), Rotary Club, Masonic Fraternity, Congregational Church, Republican Party.

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Potatoes

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INTERNAL CORK, A NEW DISEASE OF SWEET POTATO OF UNIDENTIFIED CAUSE¹

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INTRODUCTION

In the spring of 1944 the occurrence of small dark spots was observed in the flesh of a few sweet potatoes (*Ipomoea batatas* (L.) Lam., Porto Rico variety) at the Edisto Experiment Station, near Blackville, S. C., as they were being cut and examined for flesh color. At first the trouble seemed inconsequential and little attention was given to it. A few days later, however, the same trouble was noted in a stored lot of sweet potatoes near Orangeburg, S. C. Following this observation, approximately 300 stored roots were selected from Edisto Station stocks and each root was cut in thin sections and carefully examined. A trace of spotting was found in about 20 per cent of the roots. Further observations during the 1944 harvest season supported the view that a new and potentially serious disease of the sweet potato existed. The purpose of this report is to describe the symptoms of the disease, herein referred to as "internal cork," to give a preliminary account of its occurrence in South Carolina and to discuss certain unsuccessful attempts to determine its etiology.

SYMPTOMS OF INTERNAL CORK

The symptoms of internal cork are distinctive and are not likely to be confused with other types of sweet-potato flesh degeneration. The corky spots may occur singly or in groups at any point in the fleshy tissues. There are no external signs except that cavities may form at points where spots occur near the surface, as shown in figure 1, A. Numerous cases were observed, however, where spots at the periphery of the root failed to cause cavity formation (Fig. 1, B). Although many spots have been observed in the cortical zone just beneath the skin, none have ever been found erupting to form a surface lesion. Therefore, in most cases the disease can only be detected by cutting the roots and, even then, it may be overlooked unless the roots are cut in thin slices. The affected tissues, as shown in the longitudinal sections in figure 1, A, B, and D and in cross sections in C, are irregular but definite in outline. In cross section the spots may reach a diameter of about 3 cm. and rarely exceed 5 cm. in longitudinal section. Where several are grouped together, however, a considerable portion of the root may be involved. Such a case, the most severe yet noted, is shown in figure 1, D. The affected tissue is dark brown or sometimes nearly black and is hard and corky. After exposure to the air for several hours the color generally fades to medium brown. After several days' exposure, desiccation and subsequent

¹ Technical contribution number 127 of the South Carolina Agricultural Experiment Station.

shrinkage of surrounding healthy tissues gives the spots a raised or bulged appearance. Internal cork tissues remain firm during cooking. If they are crushed between the teeth, a very slight bitter taste can sometimes be detected but the flavor of the surrounding healthy tissue is not noticeably affected.



FIG. 1. Internal cork of sweet potato in roots of the Porto Rico variety. A. Longitudinal section showing a surface cavity associated with a corky area located at the periphery. B. Longitudinal section showing a peripheral spot without a cavity. C. Cross sections showing the typical random distribution of internal cork in the fleshy tissues. D. A longitudinal section of a severely affected root. Note the extensive necrotic areas which are probably aggregates of many individual spots.

Microscopic examination of sections of corky spots gave some indication of their nature. Bits of sweet-potato flesh containing corky spots were fixed in a formol-acetic-alcohol mixture, hand-sectioned, stained with Delafield's haematoxylin, and mounted in Euparal. The smallest spot examined was

composed of a necrotic area, 5 or 6 cells in diameter, located in parenchyma tissue. Larger spots (Fig. 1) showed an irregular central area of collapsed cells surrounded by a phellem layer several cells thick. Cell walls in this layer stained red with Sudan IV, which indicates presence of suberin. The remains of disorganized xylem vessels sometimes were found in the necrotic area. The time of appearance of necrosis, the exact point of its initiation or the probability of its development in storage has not been ascertained.



FIG. 2. A photomicrograph of a cross section of flesh tissue of a Porto Rico sweet-potato root, showing the marginal area of an internal cork spot. The central necrotic area, at the bottom, is separated from the healthy parenchyma tissue, at the top, by a phellem layer.

OCCURRENCE

During the 1944 harvest season samples of sweet potatoes were examined on many farms throughout the coastal plains region of South Carolina. Internal cork was found in nearly every locality visited, although it usually occurred only in trace amounts. The disease was observed in the roots of 38 different varieties and strains of sweet potatoes on trial at the Edisto Experiment Station. It was also found indiscriminately in sweet potatoes harvested from numerous experimental plots where various cultural and nutritional treatments were under investigation. A random sample of 480 roots of the Porto Rico sweet potato was collected from several fields and each root was examined and classified as to the incidence of internal cork. Thirty-nine

per cent of the roots were free from cork, 28 per cent had a trace, 25 per cent had 5 or less spots per root, and the remaining 8 per cent contained more than 5. It was judged that about half of those in the last category, or about 4 per cent, would provoke an adverse consumer reaction. The disease was observed on the trimming belts at 3 dehydration plants, although it was present in such small amounts that it apparently escaped the attention of the operators.

ETIOLOGICAL STUDIES

The possible existence of a pathogenic organism in internal cork tissues was first investigated. Forty-four stored Porto Rico roots, which showed internal cork on sectioning, were surface disinfected. Then bits of corky tissue and adjacent flesh were removed aseptically and plated on potato-dextrose agar. Forty-one of the plates remained sterile throughout a three-weeks period. The remaining 3 plates showed colonies of an unidentified *Fusarium*. According to Dr. G. M. Armstrong,² however, it is not uncommon that *Fusaria* are recovered from apparently healthy sweet-potato tissue. Similar culture of corky tissue from 35 freshly harvested roots on both potato-dextrose agar and sweet-potato-decoction agar in the fall of 1944 gave negative results in every case. Consequently the possibility that internal cork may be caused by a culturable pathogen was dismissed.

The possibility that internal cork might be due to boron deficiency was suggested by the resemblance in many respects of internal cork symptoms to those of internal black spot³ of garden beet, a boron deficiency disease. Therefore, sweet potatoes, grown in field plots which received applications of borax at rates of 0, 5, 10, 20 and 30 pounds per acre, respectively, in combination with 3 levels of potassium on both limed and unlimed soils, were examined. Although the plants had responded to the borax treatments and had symptoms ranging from severe deficiency to extreme toxicity, there was no correlation between reaction to borax and the incidence of internal cork. Inasmuch as the symptoms of sweet-potato flesh degeneration associated with a deficiency of boron⁴ were distinctly different from those of internal cork, the possibility that boron deficiency might be responsible for internal cork also was discarded.

In order to determine whether or not internal cork is transmitted from affected roots to a new crop produced by plants arising from them, several bushels of seed roots were cut in half and those with corky spots were selected for bedding in an isolated bed in the greenhouse. These seed pieces apparently produced sprouts in the normal manner. Later a duplicate plot was planted with vine cuttings taken from the original sprout-set plot. Similar plots were set adjacent to these with sprouts from an ordinary seed bed to serve as a check. All of the plots received the same fertilizer and cultural treatments. After harvest, samples were selected from each plot and exam-

² Personal communication.

³ Walker, J. C. Internal black spot of garden beet. *Phytopath.* **29**: 120-128. 1939.

⁴ Nusbaum, C. J. Internal brown spot, a boron deficiency disease of sweet potato. *Phytopath.* In press.

ined carefully for the incidence of internal cork. The disease was more than twice as prevalent in the crop originating from corky seed than from ordinary seed, as shown in table 1. Inasmuch as the internal cork status of the seed roots in the ordinary bed was not determined, these results are of doubtful significance. Nevertheless, suspicion is raised that the causal agent, whatever it may be, is root borne.

TABLE 1.—*Incidence of internal cork in Porto Rico sweet potatoes produced from sprouts and vine cuttings originating from ordinary and corky seed stocks, respectively*

Seed stock	Type of transplant	Total harvested roots exam.	Incidence of internal cork					
			None	1 ^a	2 ^b	3 ^c	4 ^d	Index ^e
Ordinary	Sprouts	No. 30	No. 15	No. 10	No. 2	No. 3	No. 0	0.77
	Vine cuttings	30	12	12	3	1	2	0.97
Corky	Sprouts	30	3	8	8	5	6	2.10
	Vine cuttings	30	0	7	9	6	8	2.50

^a Trace, only 1 spot per root.

^b Slight, 2 to 5 spots per root.

^c Weighted average of scale, none to 4, inclusive.

^d Severe, spots numerous.

^e Moderate, 5 to 10 spots per root.

Throughout the 1944 growing season sweet-potato plants, growing in various fields, were examined frequently in an attempt to discover any above-ground symptoms which could be associated with the production of internal cork affected roots. While the vines were growing vigorously through mid-season, nothing of a suspicious nature was observed. Late in the season, however, when vine growth began to decline, the older leaves near the crown of many plants showed conspicuous purplish splotches, usually from about 0.5 to 3.0 cm. in diameter and scattered promiscuously over the ventral leaf surface. The areas were irregularly circular and were usually, but not always, in a ring design with green centers. Microscopic examination of the affected portions of leaves, after clearing them in lactophenol and staining with acid fuchsin, failed to reveal any disorganization of the leaf tissues. This symptom was generally associated with the development of corky roots but numerous exceptions were found. A possible correlation between the ring spot symptom and internal cork is indicated but has not been established.

DISCUSSION

Both the origin of internal cork of sweet potato and the exact extent of its occurrence are unknown. In South Carolina it is apparently widespread, but at a relatively low level. Thus far only one inquiry concerning the disease has been received by the writer. A farmer in Union County submitted typical specimens for diagnosis.

The problem of the etiology of internal cork remains unsolved. Although only a few of the possibilities have been investigated, the behavior of the

disease thus far suggests that a virus may be involved. Preliminary attempts to transmit the disease to apparently healthy sweet-potato plants and to various other crop plants is underway.

SUMMARY

A new disease of sweet potato, referred to as internal cork was recognized for the first time in the spring of 1944. The symptoms are described. The disease was widespread but at a relatively low level in South Carolina during the 1944 season. The results here presented indicate that neither a culturable pathogen nor boron deficiency are involved and that the causal agent, as yet undetermined, may possibly be transmitted by diseased roots. A probable correlation between the late season occurrence of a ring-spot symptom appearing on the older leaves of some plants and the development of corky roots was indicated but was not definitely established. The possibility that the disease may be caused by a virus is suggested. These results are given as a preliminary report while further experimental work is in progress.

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EFFECT OF STORAGE CONDITIONS ON SURVIVAL OF *COLLETOTRICHUM GOSSYPHII*¹

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(Accepted for publication August 11, 1945)

In a study of the effect of storage conditions on the retention of viability by cotton seed the use of seed naturally infested by the anthraenose fungus, *Colletotrichum gossypii* South., afforded an opportunity to study coincidentally the survival of the infesting fungus. The results herein reported supplement the earlier data of Lehman (4) and Ludwig (5). The observed effect of the moisture content of the seed and temperature of storage should be of interest to individuals who may wish to preserve undiminished for some time the viability of fungi naturally infesting seeds. The storage conditions and their effect on the viability of the seed have been described by Simpson (6).

METHODS

For use in this storage test two lots of cotton seed naturally infested by the anthraenose fungus were obtained during the first week of October, 1937. One of the lots, Deltapine 11a, was procured from a gin at Pendleton, S. C., within 3 days after picking. The second lot, Carolinadel No. 2, was obtained from a gin at Westminster, S. C., within 10 days after picking. The initial moisture contents were 15.6 and 13 per cent, respectively. Each lot, after thorough mixing, was divided into five 100-lb. portions, and an attempt was then made to adjust these portions to moisture contents of 8, 10, 12, 14, and 16 per cent. These percentages will be used to designate the moisture contents, although the method of drying used gave actual moisture contents for the Carolinadel sub-lots of 7.2, 10.1, 12, 14.1, and 16.5 per cent; and for the Deltapine sub-lots of 7.7, 10.1, 12.1, 14, and 15.6 per cent. Additional moisture determinations were not made at the time of the tests reported in this paper. They were made, however, on 300-gm. samples which had been stored for 3 years in air-tight containers in a seed storage house at Clemson, S. C. The increases in moisture content of the samples with 7.2 to 12.1 per cent initial moisture content did not exceed 0.4 per cent. The moisture content of the sub-lots with initial contents of 14 and 14.1 per cent had increased to slightly above 15 per cent, and those at 15.6 and 16.5 per cent had increased to about 20 per cent.

To obtain moisture contents higher than the initial content, the requisite amount of water was sprinkled over the seeds, which were thoroughly mixed, then and again after 2 consecutive 24-hr. periods. In the interim and until

¹ Technical contribution No. 108 of the South Carolina Agricultural Experiment Station. Investigation made in cooperation with the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Acknowledgment is due G. W. Boozer, formerly agent of the above Division, for technical assistance in this study. This study was planned and carried out in cooperation with D. M. Simpson, U. S. Cotton Field Station, Knoxville, Tennessee.

placed in air-tight glass jars for storage, the seeds were kept in a moisture-proof container. Lower moisture contents were obtained by drying the seed in wire-bottomed trays in a drying room through which slightly heated air (not exceeding 36°C) was circulated.

In order to obtain some information concerning the most convenient means of obtaining reproducible determinations of the moisture content of cotton seed, samples of the Carolinadel lot were dried in an electrically heated oven at 80° , 90° , and 100°C . At all 3 temperatures, there were comparable very small losses up to and including the 12th day; and the mean moisture contents of the 3 samples dried at each of the 3 temperatures, if calculated from the dry weights obtained at 80° , 90° , and 100° , would have been 11.8, 12.2, and 13.7 per cent, respectively. At 90° , there was relatively little change in weight after 3 days. About 5 days were required to attain a relatively constant weight at the other 2 temperatures. Since it was impossible to judge from these data which temperature and period of drying gave the most nearly correct indication of the moisture content, a temperature of 90° for 3 days was arbitrarily chosen for drying the seed on account of the ease of obtaining a relatively constant weight at this temperature. The moisture contents given by Simpson (6) are generally slightly lower than those given in this paper, since he dried his samples at 100°C . for 24 hours and used the initial seed weights (dry weight plus water) to calculate the moisture contents.

As soon as the moisture contents were adjusted, the seeds were placed in pint jars, which were sealed with rubber gaskets. They were then sent to Simpson (6), who placed seeds of the 5 moisture contents of both lots in storage December, 1937, at about 1° , 21° , 33°C ., and the fluctuating temperatures of an unheated seed-storage house (in his paper referred to as "air temperature"), in which the seed were subjected to the range of the air temperature of Knoxville, Tenn., about -20° to 36°C . The data for this paper were obtained from samples removed from storage in December, 1938, May, 1939, and June, 1943.

To ascertain the survival of the anthraenose fungus, the seeds were germinated on water agar in test tubes, as described previously (1). A temperature of 23° – 25°C . was maintained, since this is approximately the optimal temperature for the infection of cotton seedlings by this fungus (1). Emergence of the primary roots was well advanced after 3 days, and the cotyledons began to emerge from the testas after 5 days. The elongation of the hypocotyls usually made it necessary to remove the plugs on the 9th or 10th day. Final observations were made after 12 or 14 days of incubation. A seed was considered to have germinated only when a typical primary root and hypocotyl were formed. Seeds forming primary roots only were classified as non-germinating. Since Simpson (6) used the emergence of the radicle as a criterion of viability, the percentages of viable seeds reported by him are higher than those given in this paper for several sub-lots containing seeds which formed radicles but were unable to form normal seedlings.

The appearance of typical lesions on the hypocotyls or cotyledons was used as a criterion of the survival of the fungus without any implication as to whether the absence of lesions on the seedlings was due to the death of the mycelium of the fungus or a mere loss of its ability to infect the seedlings. All diseased seedlings with lesions atypical of those caused by *Colletotrichum gossypii* were examined microscopically to ascertain the cause of such lesions. Since no seedling inoculations were attempted to ascertain whether this fungus may have survived on the seeds which did not germinate, there are no comparable data for the survival of the fungus in sub-lots with few or no viable seeds at the time of sampling. The data on seed viability have been fully discussed by Simpson (6).

RESULTS

The effect of varying moisture content of the seed on the survival of the anthracnose fungus at a given temperature is well shown in figure 1 by the percentages of infected seedlings for the sub-lots stored for 5.5 years at 1° C.

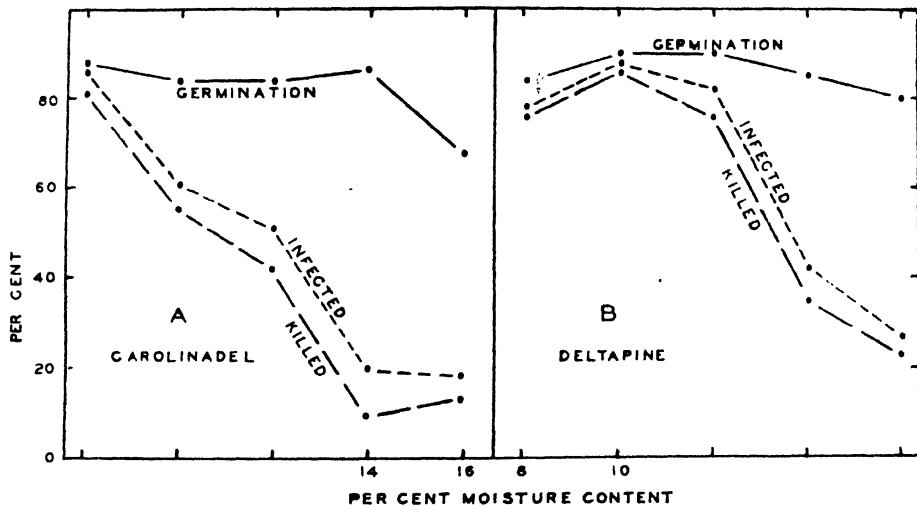


FIG. 1. Percentage germination of 2 lots of cotton seed of 8, 10, 12, 14, and 16 per cent moisture content after storage for 5.5 years at 1° C. Also number of seedlings infected and killed by the anthracnose fungus per 100 seed germinated.

For this temperature and storage period, more than 80 per cent of the seedlings of the Carolinadel sub-lot of 8 per cent moisture content and of the Deltapine sub-lots of 8, 10, and 12 per cent moisture content were infected. For the Carolinadel sub-lots, however, there were successive reductions in the number of seedlings infected and killed by the anthracnose fungus coincidentally with each increase in moisture content from 8 to 14 per cent. The Deltapine sub-lots showed a similar reduction only with the increase in moisture content from 12 to 14 per cent. The percentages of seedlings infected were about the same for the Carolinadel sub-lots of 14 and 16 per cent moisture content; but, for the corresponding Deltapine sub-lots a smaller percentage of the seedlings of the 16 per cent than of the 14 per cent moisture-content sub-lot were infected and killed.

At the higher storage temperatures of 21°, 33°, and air, the fungus infected relatively few seedlings after 17 months of storage at all 5 moisture contents. For the sub-lots which showed no loss of seed viability after this storage period, the highest percentage of infected seedlings was 20 for the Deltapine sub-lot of 8 per cent moisture content stored at 21° C. (Table 1). At air temperature, the highest infection was 14 per cent for the Carolinadel sub-lot of 12 per cent moisture content. Higher percentages of seedlings were infected for two other sub-lots, Carolinadel of 12 per cent moisture con-

TABLE 1.—*The effect of the moisture content and the temperature of storage of cotton seeds on their germination, the percentage of seedlings infected^a by the anthracnose fungus, and the percentage of seedlings killed after incubation for 12 days at 24° C. Period of storage, 17 months*

Moisture content, per cent	Storage temperature	Carolinadel No. 2			Deltapine 11a		
		Germination, per cent	Seedlings		Germination, per cent	Seedlings	
			Infected, per cent	Dead, per cent		Infected, per cent	Dead, per cent
8	1° C.	90	83	71	97	100	93
	21°	93	6	6	88	20	7
	Air ^b	90	11	4	90	11	4
	33°	90	3	2	80	9	0
10	1°	90	89	78	89	90	71
	21°	86	5	0	90	2	0
	Air	90	6	2	85	6	5
	33°	20	0	0	42	0	0
12	1°	88	85	77	90	91	70
	21°	80	13	5	75	12	0
	Air	71	14	7	83	7	0
	33°	10	0	0	0	0	0
14	1°	86	91	80	85	88	71
	21°	36	8	8	50	4	4
	Air	0	0	0	12	33	0
	33°	0	0	0	0	0	0
16	1°	88	80	73	90	81	48
	21°	0	0	0	0	0	0
	Air	0	0	0	0	0	0
	33°	0	0	0	0	0	0

^a Expressed as a percentage of the normal seedlings formed in the germination of a given sub-lot.

^b In an unheated seed storage house at Knoxville, Tennessee.

tent stored at 33° and Deltapine of 14 per cent moisture content stored at air temperature; but these two sub-lots had only 10 and 12 per cent, respectively, viable seeds.

A reduction in the survival of the anthracnose fungus was also observed in germination tests made after 12 months of storage, when the percentages of infected seedlings were about the same as those given in table 1 for 17 months of storage. The percentages of infected seedlings after 12 months are, consequently, of interest only for two sub-lots of seed which showed a distinct loss of viability by the fungus between these two dates of sampling, the Carolinadel sub-lots of 8 per cent moisture content stored at 21° and air

temperature. After 12 months of storage, these two sub-lots had 51 and 37 per cent, respectively, of the seedlings infected; and after 17 months, 6 and 11 per cent, respectively. The losses in viability of the fungus were much less for the corresponding Deltapine sub-lots with the percentages infected after 12 months being 25 and 18, respectively, and after 17 months, 20 and 11, respectively. The generally higher survival of the anthracnose fungus in this study on seeds of relatively low moisture content and stored at a relatively low temperature coincides with the observations of Lehman (4) and Ludwig (5).

The data of table 1 also indicate that the retention of seed viability was favored by relatively low seed moisture contents and temperatures. After storage for 17 months, 1° C. was the only temperature at which the seeds of all 5 moisture contents showed no reduction in viability. Similarly, the seeds of 8 per cent moisture content were the only ones to retain their initial viability at all 4 temperatures. In addition, the seed of 10 per cent moisture content showed no loss of viability at 21° and air temperature, but those of 12 per cent showed a slight loss. Thus, the viability of both the seeds and the fungus was not greatly influenced by the moisture content of seeds for a storage period of 17 months at 1° C., but the survival of the fungus was greatly reduced during this period of storage at the other temperatures and a moisture content of the seed not exceeding 10 per cent is essential for the retention of the original seed viability at 21°. These results substantiate the earlier observations of Barre (2), Lehman (4), and Ludwig (5) to the effect that the viability of the anthracnose fungus naturally infesting cotton seeds under the usual storage conditions is generally less than that of the seeds. This difference in the relative rate of the loss of viability during storage between cotton seeds and the anthracnose fungus, lead to the recommendation that seed which had been stored for 1 to 3 years be used for planting (2, 3). The necessity for the use of such seed has been superseded by the development of effective seed treatments.

Additional data that indicated a better survival of the anthracnose fungus at relatively low temperatures and low seed moisture contents were obtained when isolating fungi from lesions on seedlings grown from seeds of these sub-lots removed from storage in 1941, or after 3.5 years, and planted in a field at Knoxville, Tenn. Sixty-nine seedlings which developed from seeds stored at 1° C. and which had lesions on the hypocotyls were surface sterilized and placed on non-nutrient agar. *Colletotrichum gossypii* was secured from 43 of these seedlings, and *Fusarium moniliforme* from 20. *C. gossypii* was not found on a single one of 76 seedlings representative of those developing from seeds stored at the other temperatures. In this field the mean seedling loss² for the seeds stored at 21°, 33°, and air temperature was about 4 per cent (maximal 13.3 per cent); while the losses for the Carolinadel sub-lots of 8 to 14 per cent moisture content stored at 1° ranged from 28 to 37 per cent, and those for the Deltapine from 45 to 62 per cent. The

² Personal communication, Mr. D. M. Simpson.

losses for the sub-lots of 16 per cent moisture content stored at the same temperature were only $\frac{1}{3}$ as large as those for the sub-lots of lower moisture contents. Comparable data, previously published by Simpson (6, table 6), for a field planting in 1940 (after $2\frac{1}{2}$ years of storage) indicate a similar adverse effect of a high seed moisture content on the survival of the anthracnose fungus.

Fusarium moniliforme was the predominating fungus on non-germinating seeds, while small percentages of seed were infected by *Aspergillus* spp., a *Chaetomium* sp., an *Ophiotrichum* sp., and several other unidentified fungi. A *Rhizopus* sp. was present on the seeds of 14 and 16 per cent moisture content. All these fungi may have contaminated the samples after their removal from the storage containers. However, since they represent the typical fungus flora of non-germinating seeds, there is reason to believe that these species survived storage.

SUMMARY

Portions of 2 lots of cotton seed naturally infested by the anthracnose fungus, *Colletotrichum gossypii*, were adjusted to moisture contents of approximately 8, 10, 12, 14, and 16 per cent. Samples of each moisture content of each lot were placed in storage at 1°, 21°, and 33° C., and also at the air temperature of Knoxville, Tenn. After storage for 12, 17, and 66 months, seeds from these samples were germinated at 24° to ascertain the survival of the fungus.

The survival of the fungus, as indicated by the percentage of seedlings infected and killed, was undiminished after 12 and 17 months of storage on only the seeds stored at 1° C.

After storage for 5.5 years at 1° C., the fungus infected more than 75 per cent of the seedlings which developed from seeds stored at 8 per cent moisture content. A reduced number of seedlings tended to be infected with each successive increase in the seed moisture content up to 16 per cent, at which moisture content from 19 to 27 per cent were infected.

Under the storage conditions used, the anthracnose fungus infesting the seeds tended to lose its ability to infect seedlings before there was appreciable loss of viability by the seeds.

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THE INTERNAL INFECTION OF COTTON SEED AND THE LOSS OF VIABILITY IN STORAGE¹

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In earlier papers (1, 4) based on a cooperative study of cotton seed storage, the adverse effect of high storage temperatures and high seed-moisture contents on the longevity of cotton seed and the anthracnose fungus infesting such seed was noted. The results left unanswered the question as to whether loss of viability of the seeds in storage was due primarily to autocatalytic changes within the seeds or to infection of the seeds by micro-organisms. Both of these processes should have been favored by the higher moisture contents and by the higher storage temperatures used. The observations reported in this paper were made to ascertain whether micro-organisms were generally present in the interior of the seeds at the beginning of seed deterioration, since their presence or absence should indicate the relative rôles of infection and autocatalytic changes.

METHODS

Cotton seed from 2 storage tests were used. In one test (1, 4) made under controlled conditions, portions of 2 lots of seed, Carolinadel and Deltapine, were adjusted to approximately 8, 10, 12, 14, and 16 per cent moisture content and were then stored at 1°, 21°, 33° C., and at the air temperature of Knoxville, Tenn. The other seeds were obtained from a cooperative locality-of-storage test, supervised by D. M. Simpson, in which portions of a lot of Stoneville-2b seed were stored in ordinary seed houses at 7 localities in the southern United States, as listed in table 1. One-half of each original lot of seed was acid-delinted, and the 4 bags of seed stored at each locality were: (a) fuzzy seed not treated, (b) fuzzy seed treated with 2 per cent Ceresan, (c) acid-delinted seed, and (d) acid-delinted seed treated with 2 per cent Ceresan.

If germination tests made by Simpson indicated loss of viability by a given sub-lot of seed, samples were sent the writer. Individual seeds were cultured in test tubes on sterile water agar at 24° C. (1). In order to remove all external contamination by micro-organisms before culturing, the seeds not previously delinted were acid-delinted, washed in tap water, and air-dried. Immediately before they were placed in the test tubes, all seeds were immersed for 2 minutes in a solution of HgCl_2 in 50 per cent ethanol (2.5 gm./l.), and were then washed with sterile water. One hundred seeds were cultured for each sample and were observed for at least 2 weeks.

¹ Technical contribution No. 122 of the South Carolina Agricultural Experiment Station. Investigation made in cooperation with the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Acknowledgment is due G. W. Boozer, formerly agent of the above Division, for technical assistance in this study. This study was planned and carried out in cooperation with D. M. Simpson, U. S. Cotton Field Station, Knoxville, Tennessee.

The development of a seed into a seedling with a normal primary root, hypocotyl, and cotyledons was used as a criterion of normal germination and correspondingly of a normal viable seed. The loss of viability by the seeds was associated with the appearance of abnormal seedlings which have been grouped into 2 classes to facilitate the presentation of the data: (a) those

TABLE 1.—*Germination of a lot of Stoneville-2b cotton seed after storage for 20 months in 7 different localities. Data are based on the germination of 100 seeds*

Location	Kind of seeds	Kinds of seedlings from viable seeds				Nonviable seeds	
		Normal	Normal tops, abnormal radicles	No tops, abnormal radicles		Total	Infected ^b
				Total	Infected ^b		
Clemson, S. C.	FU	86	2	12		0	
	FT	62	30	5		3	
	DU	88	0	12		0	
	DT	70	0	17		13	
Jackson, Tenn.	FU	85	0	8	6-Pen.	7	2-Bact.
	FT	70	0	22		8	
	DU	60	2	20	2-Asp.	18	
	DT	63	2	35		0	
Knoxville, Tenn.	FU	70	0	17	2-Fus.	13	8-Bact.
	FT	72	0	12		16	3-Bact.
	DU	70	0	12		18	5-Bact.
	DT	55	10	12		23	8-Bact.
Greenville, Texas	FU	88	2	0		10	
	FT	60	0	27		13	3-Bact.
	DU	57	3	30		10	
	DT	55	5	40		0	
State College, Miss.	FU	50	17	22		11	2-Bact.
	FT	50	5	40		5	
	DU	65	2	28	2-Asp.	5	2-Pen.
	DT	10	5	47		38	8-Bact.
Florence, S. C.	FU	20	22	24		34	10-Bact.
	FT	38	10	35		17	3-Pen.
	DU	8	2	32		58	12-Bact.
	DT	30	5	50		15	
Baton Rouge, La.	FU	8	5	65		22	2-F., 3-Bact.
	FT	5	10	53		32	
	DU	10	5	33		52	10-Pen., 2-Asp., 8-Bact.
							2-Bact.
	DT	2	5	43		50	

^a FU, fuzzy seed not treated; FT, fuzzy seed treated with Ceresan; DU, acid-delinted seed, not treated; DT, acid delinted seed treated with Ceresan.

^b Abbreviations following the numbers refer to species of *Penicillium*, *Aspergillus*, *Fusarium*, to a nonsporulating fungus (F.), or to bacteria.

that formed short radicles but no evident hypocotyls and whose cotyledons did not emerge from the testas; and (b) those that formed normal hypocotyls and cotyledons but did not form primary roots of normal length. The abnormal primary roots were usually from 3 to 6 cm. long, and their apices were blunt and dark. When normal cotyledons developed on such seedlings

numerous secondary roots were generally formed at the upper portion of the radicle and the base of the hypocotyl, which permitted the continued growth of the seedlings. Total germination is used to designate the percentage of seeds that formed radicles, regardless of the normality of subsequent development, and is equivalent to the ordinary usage of this term in germination tests. The various kinds of abnormal seedlings produced by the sub-lots, after a loss of viability was evident, are illustrated in figure 1.

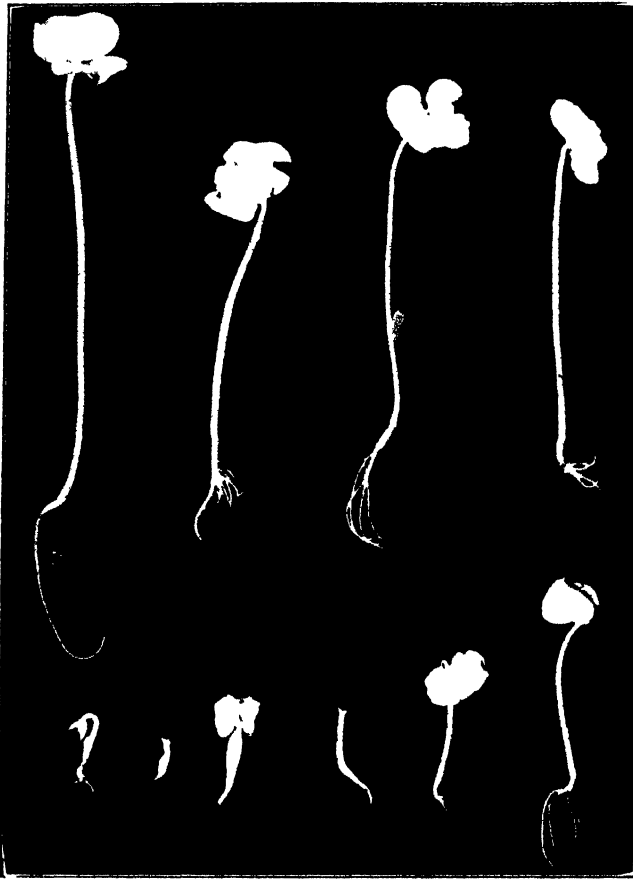


FIG. 1. Abnormal seedlings produced by cotton seed of 12 per cent moisture content after storage for 28 months in a seed house at Knoxville, Tenn. Seedlings photographed after 10 days' incubation at 24° C. Normal seedling, upper left.

RESULTS

There were large differences in the rate of seed deterioration among the 7 localities in which the Stoneville lot was stored (Table 1). The conditions that may have been responsible for these differences will be discussed by Simpson in a later paper. The differences in rates of deterioration among the sub-lots of seed of several moisture contents stored at different temperatures were clearly related to both moisture content and temperature. Via-

bility was lost more rapidly, in 4 months, in the lots of 16 per cent moisture content stored at 33° C. (Table 2); while for seed of 8 per cent moisture content stored at the same temperature, the viability was undiminished after 5.5 years (1). The data of table 2 indicate an increase in the rate of deterioration with each successive increase in the moisture content and temperature for lots which are otherwise comparable. One sub-lot, B-10-33 of table

TABLE 2.—*Percentage germination and types of seedlings obtained from two lots of seed of five different moisture contents after storage at different temperatures*

Variety, moisture content, temperature, and length of storage ^a	Germination and types of seedlings ^b			
	Total	Normal seedlings	Normal tops, abnormal radicles	No tops, abnormal radicles
A-16-33-4	0	0	0	0
B-16-33-4	0	0	0	0
A-14-33-4	65	28	18	19
B-14-33-4	1	0	0	1
A-12-33-4	89	74	7	8
A-12-33-12	50	29	9	12
B-12-33-4	81	50	10	21
B-12-33-12	54	28	7	19
B-12-33-17	0	0	0	0
A-10-33-12	87	87	0	0
A-10-33-17	72	16	18	38
B-10-33-12	60	45	9	6
B-10-33-17	74	5	20	49
A-16-air-12	0	0	0	0
B-16-air-12	0	0	0	0
A-14-air-12	39	23	8	8
A-14-air-17	0	0	0	0
B-14-air-12	48	26	19	3
B-14-air-17	18	0	0	18
B-14-air-28	0	0	0	0
A-12-air-28	45	8	17	20
B-12-air-28	47	14	13	20
A-16-21-12	0	0	0	0
B-16-21-12	35	15	12	8
A-14-21-17	26	0	0	26
A-14-21-28	0	0	0	0
B-14-21-17	71	50	0	21
B-14-21-28	41	3	8	30
B-12-21-17	95	89	0	6
A-12-1-28	88	86	2	0
B-12-1-28	90	87	0	3

^a Variety A is Carolinadel; variety B is Deltapine. A-16-33-4 designates a seed lot of Carolinadel of 16 per cent moisture content stored at 33° C. for 4 months. "Air" refers to the temperature of a seed house at Knoxville, Tenn.

^b Based on 100 seeds.

2, shows an apparently higher germination after 17 than after 12 months of storage, but the percentage of normal seedlings was smaller after the longer storage period. When there was little or no loss of viability, the percentages of normal seedlings for the sub-lots of table 2 approximate the percentages of germination based on radicle protrusion reported by Simpson (4); but, after deterioration was evident, the percentages reported by Simpson approximate the sum of the normal and abnormal seedlings of table 2.

The concurrent increase in the percentage of abnormally germinating seeds accompanying a loss of viability may be indicated by grouping the sub-lots of table 2 into 4 groups with the total percentage of viable seeds (a) more than 80, (b) between 60 and 80, (c) between 40 and 60, and (d) less than 40. For these 4 groups, the percentages of normal seedlings were 90, 42, 38, and 32, respectively; the percentages of embryos that formed tops but had abnormal radicles were 3, 19, 26, and 17, respectively; and the percentages of embryos that formed only short radicles were 7, 39, 36, and 51, respectively. The same increase in the percentage of abnormal seedlings was also shown by individual sub-lots. Thus, for sub-lot A-10-33 during the interval between sampling after 12 and 17 months, the total germination decreased from 87 to 72 per cent; while the percentage of seedlings with abnormal roots increased from 0 to 18 and the percentage which formed only radicles increased from 0 to 38.

The small percentages of seedlings infected by micro-organisms indicated that infection of the seeds was not the primary cause of the loss of viability. Less than 2 per cent of the seedlings obtained in the germination of seeds from the controlled-condition storage test were infected; and these were all seedlings with dark, blunt apices, which were infected by a *Penicillium* sp. In the locality-of-storage test only 12 seedlings were infected, and these were abnormal seedlings with short radicles and without hypocotyls and cotyledons that had developed from seeds that had not been treated with Ceresan (Table 1).

The percentages of nonviable seeds which were internally infected were much higher than the percentages for the viable seeds, but generally not all were infected, as shown in table 3 for sub-lots A-10-33-12, A-10-33-17, B-10-33-12, A-14-air-12, and B-14-21-17. Four of the sub-lots with no viable seed had practically all seeds infected; while a fifth lot, A-16-21-12, had only 68 per cent infected. The percentages of infected nonviable seeds were generally smaller in the locality-of-storage test than in the controlled-condition test, only 19 per cent of the 491 nonviable seeds of the former being infected (Table 1). The treatment of the seeds with Ceresan before storage did not prevent the infection of these seeds by fungi and bacteria, although a smaller percentage of the treated than of the untreated seeds was infected.

The Deltapine sub-lot of 14 per cent moisture content stored at 21° C. illustrates the increase in percentage of nonviable seeds infected with an increased length of storage. After 17 months of storage only 7 per cent of the seed were infected (no infection of viable seeds), but after an additional 11 months of storage 50 per cent were infected. Similar increases are evident for the sub-lots A-12-33, A-14-air, B-14-air, and A-14-21.

The predominating fungus obtained from the infected nonviable seeds was a white-spore species of *Aspergillus*. Yellow- and black-spore species of this genus were less abundant. The genus *Penicillium* was represented by several species; a grayish-spore species was found most frequently, with yellow- and green-spore species found less frequently. As indicated in table

3, *Diplodia theobromae* was found only in the Carolinadel sub-lot of 16 per cent moisture content after 4 months storage at 33° C., when 40 per cent were infected. This lot of seed was infested externally by this fungus at the start of the experiment. Its failure to infect the nonviable seeds of the other sub-lots indicates that a relatively high temperature and a high moisture

TABLE 3.—Percentage of non-viable seeds for the two lots of cotton seed listed in table 2 and the number of seeds per 100 seeds cultured that were infected by several species of fungi and bacteria

Variety, moisture content, temperature, and length of storage ^a	Percentage non-viable seed	Number of seeds infected by micro-organisms				
		Total	Bacteria	<i>Aspergillus</i> spp.	<i>Penicillium</i> spp.	Other fungi ^b
A-16-33-4	100	100	10	20	30	40-Dip.
B-16-33-4	100	96	0	93	3	0
A-14-33-4	35	19	3	3	12	1-Col., 1-F.
B-14-33-4	99	96	0	98	8	0
A-12-33-4	11	7	1	0	5	1-F.
A-12-33-12	50	28	6	12	4	6-F.
B-12-33-4	19	6	0	0	4	2-F.
B-12-33-12	46	20	6	4	6	4-F.
B-12-33-17	100	33	33	0	0	0
A-10-33-12	13	0	0	0	0	0
A-10-33-17	28	10	10	0	0	0
B-10-33-12	40	14	6	2	4	2-F.
B-10-33-17	26	16	11	2	1	2-F.
A-16-air-12	100	68	54	8	6	0
B-16-air-12	100	66	46	12	6	0
A-14-air-12	61	22	16	0	2	2-Col., 2-F.
A-14-air-17	100	80	80	0	0	0
B-14-air-12	52	22	20	2	0	2-Col.
B-14-air-17	82	50	50	0	0	0
B-14-air-28	100	94	94	0	0	0
A-12-air-28	55	49	49	0	0	0
B-12-air-28	53	53	53	0	0	0
A-16-21-12	100	68	54	8	6	0
B-16-21-12	65	12	20	22	0	0
A-14-21-17	74	45	45	0	0	0
A-14-21-28	100	97	97	0	0	0
B-14-21-17	29	7	7	0	0	0
B-14-21-28	59	52	50	0	0	0
B-12-21-17	5	4	2	0	0	0
A-12-1-28	12	7	7	0	0	1-Col.
B-12-1-28	10	8	6	0	0	2-Fus.

^a See footnote to table 2.

^b Abbreviations following the numbers refer to *Diplodia theobromae*, *Colletotrichum gossypii*, species of *Fusarium*, or to a nonsporulating fungus (F.).

content are essential for the infection of the seed by this fungus. When first stored (1) both of these lots of seed were also infested by several species of *Fusarium* and by *Colletotrichum gossypii*. The results (Table 3) show that the latter fungus was obtained from only 6 seeds after deterioration of the seed had started; and Fusaria were obtained from only 2 seeds. Apparently, infection of seeds by these fungi did not increase after storage or they were overgrown by various saprophytes. The seeds which were infected by fungi

also were invariably infected by several species of bacteria, of which the most common was a species which formed white colonies on agar. This same species was also the most prevalent of the bacteria which infected the seeds when they were not infected by fungi.

DISCUSSION

The failure to demonstrate infection, by micro-organisms, of most of the abnormal seedlings and many of the nonviable seeds seems to indicate that the initial loss of viability was probably associated with autocatalytic processes within the embryo itself, as described by Katon and Altschul (2). Since the meristem of the primary root was the first to lose its capacity for indefinite growth, this portion of the embryo is apparently more susceptible to injury by such processes than the other meristems. Similar injury has been noted in other studies in which seeds that had been stored in the laboratory were germinated in sand culture. The first evidence of reduced viability was delayed emergence of a portion of the seedlings. The apices of the radicles of these seedlings were invariably blunt and dark. These seedlings, in part, formed secondary roots at the base of the hypocotyl and upper portion of the primary root (Fig. 1), and they developed into normal plants. The more severely affected primary roots grew in almost any direction (at times even emerging from the sand) as if they had lost their capacity for normal geotropic response.

Although the data indicate that internal infection of the seeds by bacteria and fungi could not have been the primary cause of the loss of viability of the seeds under the storage conditions of these studies, this does not apply necessarily to the sub-lots of 14 and 16 per cent moisture content stored at 33° C. which lost their viability during the first 4 months of storage, and which were almost 100 per cent infected at the time of the first examination.

The technique used in these studies did not eliminate the possibility that in certain instances the loss of viability may have been accelerated by the action of fungi and bacteria on the peripheral portions of the seed, if it may be assumed that the surface treatment of the seeds with H_2SO_4 and the HgCl_2 solution killed or inhibited the growth of such fungi and bacteria, when the seeds were cultured on water agar. This possibility seems to be definitely eliminated by the fact that the deterioration of the Ceresan-treated sub-lots in the locality-of-storage test was not greatly different from that of the untreated seed. Similarly, Robertson *et al.* (3) found that there was little difference between the rate of deterioration of untreated and Ceresan-treated barley, oats, and wheat seed which had been stored at several relative humidities.

SUMMARY

Cotton seed in which there was a reduction of viability after storage were surface-sterilized and germinated under sterile conditions. In one study, seeds of 5 moisture contents were stored at 4 temperatures (1°, 21°, 33° C., and that of Knoxville, Tennessee) for periods of 4, 12, 17, and 28 months. In another study, seeds were stored in 7 localities in ordinary storage houses.

A negligible percentage of the viable seeds, including those that formed abnormal seedlings, were infected by bacteria or fungi, and many of the non-viable seeds were not infected, which indicates that micro-organisms are not necessarily the primary cause of the deterioration of cotton seed at ordinary storage temperatures when the initial moisture content of the seed is 14 per cent or less.

The onset of the loss of viability by the seeds was associated with the appearance of abnormal seedlings. The various abnormalities indicated that the meristem of the primary root was the first portion of the embryo to be injured during storage and to lose its capacity for indefinite growth, although it generally retained its capacity for limited growth longer than the meristem of the hypocotyl. The primary roots of abnormal seedlings rarely developed beyond a length of 4 to 6 cm.; and their apices were generally blunt and discolored.

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36847

SOME FACTORS INFLUENCING CURLY TOP VIRUS CONCENTRATION IN SUGAR BEETS

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INTRODUCTION

In earlier studies on susceptibility of sugar beet to the curly-top disease (1) there was evidence suggesting that the virus concentration in infected resistant beets used as virus sources was lower than in infected plants of the more susceptible varieties. It often happened that an appreciably lower percentage of test plants became infected when the vectors were leafhoppers that had fed on diseased, resistant plants than when similar vectors had fed on diseased, susceptible plants. This was not given much consideration at that time because it was recognized that there were many other factors which influence the amount of infection among test plants. As highly resistant varieties of sugar beet were developed it appeared more important to determine the relationship between resistance and virus concentration.

In 1929, Holmes (5) reported a convenient and accurate method of determining the relative concentrations of tobacco mosaic virus, but no similar "local lesion" method for use with the curly-top virus, *Ruga verrucosa* Carsner and Bennett, has been discovered. Lackey (6) reported that root tips of curly-top infected sugar beets and of beans had a higher virus concentration in the portion below the protophloem sieve tubes than in portions just above that region. Using virus strain 1, he reported (7) that the root tips of resistant sugar beets had a higher virus concentration than those of the susceptible beet. He also reported (8) that root tips of the susceptible bean variety Bountiful had a higher virus concentration when infected with the more virulent strain 1 than when infected with the less virulent strain 4, but that in root tips of susceptible beets the less virulent strain 4 reached a somewhat greater concentration than strain 1. In all of these experiments he fed nonviruliferous leafhoppers on juice from the root tips to be tested and then caged them on young, susceptible sugar-beet plants, using one leafhopper to a plant.

METHODS

Nonviruliferous leafhoppers were fed for a short period upon plants infected with a known virus strain and hereafter designated as "source plants." They were then transferred to healthy, young sugar-beet plants, one leafhopper being caged on each test plant. The percentage of plants which became infected from each source plant was determined and it was assumed that such infection data gave good evidence as to relative concentrations of curly-top virus.

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Young adult leafhoppers have been more satisfactory than nymphs or old adults. Nymphs were sometimes found moulting in the cages on source plants and at this stage they may not feed for some hours. The older adults were likely to feed less than active nymphs or young adults and to die in greater numbers than the young adults.

The sugar-beet varieties used as source plants were the highly resistant S.L. 68 and the very susceptible S.L. 842. The curly-top virus strains used were those previously described (2) as strain 1 and strain 2. Each of these strains readily infects the resistant beet but neither of them induces severe injury in this host. Plants of the resistant S.L. 68 infected by strain 1 show vein clearing, occasional small papillae along the veins on the underside of the leaves and in many cases some rolling of the leaves (See (1R) in figure 1). Plants of S.L. 68 infected by strain 2 show similar but less obvious



FIG. 1. Typical curly top symptoms on young sugar beets 30 days after inoculation: beet variety S.L. 842 infected by virus strain 1 (1), beet variety S.L. 68 infected by virus strain 1 (1R), and beet variety 842 infected by virus strain 2 (2).

symptoms. It is often necessary to look rather closely at plants of this resistant variety infected with strain 2 to detect any symptoms. Plants of the susceptible variety S.L. 842 are readily infected by either virus strain. In such plants infection by strain 1 causes extreme dwarfing and distortion of the foliage, while infection by strain 2 induces relatively little distortion or injury (Fig. 1). Symptoms induced in plants of the susceptible variety by strain 2 were similar to those induced in plants of the resistant variety by strain 1. The source plants selected for use were as nearly comparable as practicable but differences in severity of symptoms were unavoidable.

Feeding periods of nonviruliferous leafhoppers on source plants were varied from 15 minutes to 72 hours. For feeding periods of ten hours or less the leafhoppers were caged on the source plants during the day. In the earlier experiments the source plants were kept in an auxiliary greenhouse where the temperature range might be anywhere from 70° to 100° F. A high temperature and a relatively short feeding period on the source plants appeared to give better results than longer feeding periods at lower temperatures, so the last seven experiments were conducted with the source plants in a special feeding chamber where it was possible to maintain a

temperature of about 100° F. A feeding period of two or three hours was found adequate under these conditions.

Leafhoppers which had fed on the source plant to be tested for virus concentration were transferred to young sugar beet plants in the early two-leaf stage and permitted to feed on these for a week at ordinary greenhouse temperatures ranging from 60° to 90° F. Each test plant was inoculated through the cotyledon, using a small leaf cage and one leafhopper to a plant.

The curly-top susceptible beet S.L. 842 was used as a test plant in every experiment and the highly resistant beet S.L. 68 was also used as a test plant in a number of experiments. All test plants were set out in boxes 22½ inches long by 5½ inches wide by 4¾ inches deep, inside dimensions, with 10 or 12 plants arranged as five or six pairs lengthwise of the box. Inoculations were made in pairs so that each virus source occurred on plants adjacent to two other sources which were to be compared with it and so that inoculations from the same source did not always occur at the same position in the box. This gave something approaching randomized arrangement of the test plants with different virus sources and assured comparable results.

EXPERIMENTAL WORK

The method used for determining virus concentration cannot be considered very exact. In view of this, and since there is abundant evidence of differences in virus concentration of individual source plants, it seems best not to include detailed data covering each of the experiments.

Report on a Typical Experiment. The detailed data from a representative experiment are given in table 1. This table gives data to indicate relative concentration of curly-top virus in resistant source plants and in susceptible source plants. Plants 1 and 2 are considered as one pair of source plants, 3 and 4 as another pair, and so on. The concentration of virus strain 1 was tested from two pairs of source plants and for three periods of feeding, making six paired tests. The concentration of virus strain 2 was tested from three pairs of source plants and for three periods of feeding, making nine paired tests. Each of these tests was planned to include 24 plants and variation from that number was due to death of an occasional plant. This experiment included the inoculation of 714 test plants from 10 source plants.

Relative virus concentrations in plants infected by strain 1 and in those infected by strain 2 are revealed in table 1 by considering plants 1 and 5 as one pair, 3 and 7 as another, 2 and 6 as another, and 4 and 8 as another. Plants 9 and 10 cannot be considered for strain comparison as they were each infected with strain 2. The data for this comparison were secured from 571 test plants and from 8 source plants. In this case some data for comparing concentrations of strains 1 and 2 were secured incidentally from an experiment set up primarily to determine possible virus concentration differences between the resistant and the susceptible source plants. Many experiments were set up for the single purpose of comparing resistant source plants and susceptible source plants. Other experiments involved only

TABLE 1.—*Typical summary sheet from curly-top virus concentration experiments. Data from tests of March 3, 1939, including 15 tests which cover 5 pairs of source plants, 3 periods of feeding, and 2 strains of virus. Tests made upon young plants of susceptible sugar-beet variety S.L. 842*

Leafhopper feeding period on source plants	Virus from susceptible source plants					Virus from resistant source plants				
	Source plant numbers	No. of plants inoc.	Plants diseased	Average incubation period	Source plant numbers	No. of plants inoc.	Plants diseased		Average incubation period	Days
							Number	Percent		
3 hrs.	1	23	12	52	2	24	8	33	9.1	9.1
	3	24	15	63	4	24	13	54	11.2	11.2
	5	23	12	52	6	24	7	29	10.1	10.1
	7	24	4	17	8	24	11	46	11.1	11.1
	9	24	13	54	10	24	7	29	11.4	11.4
6 hrs.	1	24	18	75	2	23	14	61	9.7	9.7
	3	24	16	67	4	24	17	71	9.7	9.7
	5	23	17	74	6	24	11	46	13.4	13.4
	7	24	10	42	8	24	13	54	11.2	11.2
	9	24	13	54	10	24	8	33	11.6	11.6
9 hrs.	1	24	18	75	2	24	14	58	11.1	11.1
	3	24	19	79	4	24	15	63	9.8	9.8
	5	24	21	88	6	23	16	70	11.1	11.1
	7	24	11	46	8	24	15	63	12.9	12.9
	9	24	18	75	10	23	12	52	12.8	12.8

^a Source plants 1 through 4 had been inoculated January 9, 1938, with virus strain 1. Source plants 5 through 10 had been inoculated January 9, 1939, with virus strain 2.

comparisons of source plants infected by strain 1 with plants infected by strain 2.

Individual variation in the source plants is indicated by plant 7 in table 1 which showed the lowest virus concentration of any of the susceptible source plants for each of the three feeding periods. Plant 10 gave similar evidence as a resistant source. Plant 7 happened to be paired against a resistant source plant, No. 8, which consistently showed a relatively high virus concentration for strain 2 so that in each of the three feeding periods the resistant source plant indicated a higher concentration than the susceptible.

The data in table 1 also indicate that plants infected by virus strain 1 contained a higher concentration of virus than those infected by strain 2. This was true in both the resistant group of source plants and the susceptible group. Both the percentages of plants infected and the periods of incubation suggest a greater virus concentration for strain 1. Another possibility would be that the longer incubation period for strain 2 is due to a slower response on the part of the host. The incubation period was not used as a factor in determining the significance of data dealing with relative concentrations.

The six paired tests involving strain 1 show only one case, plants No. 3 and 4 in the six hour period, in which the resistant source plant indicated a higher virus concentration than the susceptible. In five of these six tests the incubation period was longer for plants infected from the resistant source plants than for those infected from the susceptible source plants. The nine paired tests involving strain 2 showed three instances in which the resistant source plant indicated a higher virus concentration than the susceptible and the same pair of source plants, 7 and 8, were involved in each case. The susceptible source plant carried a higher virus concentration in the other six tests. Such data from one experiment do not indicate significant differences, but similar results from many experiments were highly significant when subjected to the χ^2 test for significance.

Relative Concentration as Influenced by Resistance of Beet. The highly resistant sugar beet variety S.L. 68 and the susceptible variety S.L. 842 were used as virus-source plants. The tests were conducted with curly-top virus strains 1 and 2. Each of these strains readily infects either the susceptible or the resistant sugar beet but strain 1 induces relatively much more severe symptoms than strain 2.

The tests on susceptible plants from source plants carrying strain 1 showed that the susceptible source plants contained a higher virus concentration than the resistant source plants in a very large percentage of the tests (Table 2). On susceptible test plants there was 11 per cent more infection among the total of 1,673 inoculated with strain 1 from susceptible source plants than among the total of 1,667 inoculated from resistant source plants (Table 3).

Tests on resistant plants from source plants carrying strain 1 gave a still larger percentage of tests in which the susceptible source plants showed the

TABLE 2.—*Consolidated data from tests to determine the relative curly-top virus concentrations in resistant and in susceptible sugar-beet plants^a*

Source of virus and susceptibility of test plants	Source plant pairs	Feeding periods	Total tests	Tests showing					
				No difference in virus concentration		Lower virus concentration in susceptible source plant			
				Number	Percent	Number	Percent		
Source plants infected with strain 1 virus									
Test plants susceptible	35	27	74	5	7	15	20	54	73
Test plants resistant	13	9	30	2	7	7	18	30	77
Source plants infected with strain 2 virus									
Test plants susceptible	8	9	24	0	0	4	17	20	83
Test plants resistant	3	3	9	0	0	0	0	9	100

^a Resistant source plants were the sugar-beet variety S.L. 68 and the susceptible source plants were the variety S.L. 842.^b Each pair of source plants consisted of one resistant sugar beet and one susceptible sugar beet, each infected with the same strain of virus and otherwise comparable.

higher virus concentration (Table 2). On resistant test plants there was 11 per cent more infection among the total of 890 inoculated from susceptible source plants than among the total of 893 inoculated from resistant source plants (Table 3).

This 11 per cent difference between the amount of infection from susceptible source plants and the amount from resistant source plants is the same as the difference shown when susceptible source plants were used but the eleven point increase over 23 per cent in the case of resistant test plants is nearly one-half, whereas the same increase over 54 per cent in the case of susceptible test plants is only one-fifth. The infection percentages secured from resistant source plants and from susceptible source plants show very different ratios depending upon whether the test plants are resistant or susceptible. Similar differences are evident in all experiments involving the resistant and the susceptible source plants.

TABLE 3.—*Summary of results of test-plant inoculations made from resistant and from susceptible sugar-beet plants during studies to determine relative curly-top virus concentrations**

Source and strain of virus	Resistant test plants			Susceptible test plants		
	Inoculated			Inoculated		
	Num- ber	Num- ber	Per cent	Num- ber	Num- ber	Per cent
Susceptible plants carrying strain 1	890	305	34	1673	1085	65
Resistant plants carrying strain 1	893	202	23	1667	905	54
Susceptible plants carrying strain 2	564	331	59	565	407	72
Resistant plants carrying strain 2	215	26	12	213	69	32

* The resistant sugar beet was the variety S.L. 68 and the susceptible beet was the variety S.L. 842.

Comparative tests for virus concentration in the susceptible beet and in the resistant beet when infected by virus strain 1 are somewhat complicated by the fact that the symptoms are different in the two beet varieties. The susceptible beet is greatly dwarfed and distorted whereas the resistant beet is not dwarfed and has relatively mild symptoms (Fig. 1). The extreme dwarfing of the susceptible plants means that the available tissue for leafhoppers to feed upon is much less than in the case of an infected, resistant plant of the same age. This might mean less favorable feeding conditions due to crowding of the leafhoppers.

Susceptible sugar beet plants infected with curly-top virus strain 2 do not show the dwarfing and distortion mentioned in connection with strain 1 and so might be thought to give a more accurate picture as to the relative concentrations. Tests on susceptible plants from source plants carrying strain 2 give a still greater percentage in which the susceptible source plant carried a higher virus concentration than the resistant source plant (Table 2). Of the total susceptible test plants inoculated there was 40 per cent more infection among those inoculated from susceptible source plants than among those inoculated from the resistant source plants (Table 3).

TABLE 4.—Consolidated data from tests to determine the relative early top virus concentrations in plants infected by strain 1 and in plants infected by strain 2^a

Source of virus and susceptibility of test plants	Source plant pairs ^b	Feeding periods	Total tests		No difference in virus concentration		Tests showing			
			Number	Percent	Number	Percent	Lower virus concentration for strain 1		Lower virus concentration for strain 2	
	Number	Number	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Susceptible source plants										
Susceptible test plants	21	9	61		5	8	17	28	39	64
Resistant test plants	17	0	47		3	6	8	17	36	77
Resistant source plants										
Susceptible test plants	10	9	30		2	7	5	17	23	77
Resistant test plants	6	6	18		0	0	0	0	18	100

^a According to the amount of injury induced on susceptible and on resistant sugar beets, strain 1 is highly virulent while strain 2 has relatively low virulence.

^b Each pair of source plants consisted of one sugar beet infected with strain 1 and one infected with strain 2. The two plants were of the same sugar-beet variety and otherwise comparable.

Tests on resistant plants from source plants carrying strain 2 showed a higher virus concentration for the susceptible source plant in every instance (Table 2). Of the total resistant test plants inoculated there was 47 per cent more infection among those inoculated from susceptible source plants than among those inoculated from the resistant source plants (Table 3).

The difference in virus concentration was indicated more strikingly when resistant test plants were used than when susceptible test plants were used. This was true whether considered on the basis of tests made or on the actual number of test plants inoculated and for both virus strains 1 and 2. It is also evident that the difference in concentration was greater, or that the

TABLE 5.—*Summary of results of test-plant inoculations made from sugar-beet plants infected with strain 1 or with strain 2 during studies to determine relative curly top-virus concentration**

Source and strain of virus	Resistant test plants			Susceptible test plants		
	Inoculated			Inoculated		
	Num- ber	Num- ber	Per cent	Num- ber	Num- ber	Per cent
Strain 1 from susceptible plants	958	242	25	1282	661	52
Strain 2 from susceptible plants	948	100	11	1282	530	41
Strain 1 from resistant plants	372	65	17	664	358	54
Strain 2 from resistant plants	379	20	5	664	267	40

* According to the amount of injury induced on susceptible and on resistant sugar beets, strain 1 is highly virulent while strain 2 has relatively low virulence. Data in this summary are not comparable with data in table 3. The data for strain 1 virus from susceptible source plants on resistant test plants are typical of each group. The leafhopper vectors used on 10 per cent of the 958 plants (Table 5) had fed for 6 hours to 48 hours on source plants and 90 per cent of them had fed for 3 hours or less on source plants, whereas the vectors used on 61 per cent of the 890 plants (Table 3) had fed for 6 hours to 48 hours on source plants while only 39 per cent had fed for 3 hours or less on source plants. This resulted in a lower percentage of infection for the test plants in table 5 and it would be unfair to compare percentages from susceptible and from resistant source plants in this table with those in table 3. Most of the experiments included in the 156 tests involving the data in table 5 were set up under conditions which would not warrant the use of any data from them in connection with table 3.

difference was brought out more decidedly when strain 2 was used than when strain 1 was used.

The evidence shows conclusively that resistant sugar beet plants carry a much lower concentration of curly-top virus than similar plants of a susceptible variety when both are infected with the same virus strain.

Virus Concentration as Related to Virulence of the Virus Strain. Any comparison of strains 1 and 2 from susceptible source plants involves the consideration of the differences in severity of symptoms. It is believed that efforts to secure a fair comparison in the case of susceptible source plants were successful, and it is certain that dwarfing and distortion were not involved as factors when the resistant beet S.L. 68 was used as a source plant.

Tests on susceptible plants from susceptible source plants showed a greater virus concentration for plants infected with strain 1 than for those infected with strain 2 in a high percentage of the tests (Table 4). Of the

total susceptible test plants inoculated there was 11 per cent more infection among those inoculated from strain 1 source plants than among those inoculated from strain 2 source plants (Table 5).

Tests on resistant plants from susceptible source plants showed a still greater percentage of cases in which there was higher virus concentration for the plants carrying strain 1 (Table 4). Of the total resistant test plants inoculated there was 14 per cent more infection among those inoculated from strain 1 source plants than among those inoculated from strain 2 plants (Table 5).

Tests on susceptible plants from resistant source plants showed a higher virus concentration for plants carrying strain 1 than for those carrying strain 2 (Table 4). Of the total susceptible test plants inoculated there was 14 per cent more infection among those inoculated from strain 1 source plants than among those inoculated from strain 2 source plants (Table 5).

Tests on resistant plants from resistant source plants showed a higher virus concentration for the strain 1 source plant in every case (Table 4). Of the total resistant test plants inoculated there was 12 per cent more infection among those inoculated from plants carrying strain 1 than from those carrying strain 2 (Table 5).

When comparing strains 1 and 2 it again appears that differences in virus concentration were more clearly evident when the test plants were of the resistant beet variety than when the susceptible beet was used. This was true whether the source plant was susceptible or resistant, but the differences were more striking on either variety of test plant when the source plant was resistant. This might indicate an actually greater difference in concentration in the resistant source plants or that any existing difference is more strikingly brought out.

It is clearly evident that sugar-beet plants infected with strain 1 carry a higher virus concentration than those infected with strain 2.

Virus Concentration as Related to Time Elapsed after Infection. A sugar-beet plant which has been infected by curly top either dies in a few weeks or shows a more or less pronounced tendency to recover as evidenced by less severe symptoms on the new growth. This is true either in the field or in the greenhouse.

The source plants used in these tests were inoculated in the young two-leaf stage. Some plants which had been infected for 24 days were compared with others which had been infected for 5 months; plants infected for 5 weeks were compared with others infected for 5½ months; plants infected for 2 months were compared with others infected for 8 months; and some infected for 3 months were compared with others infected for 7 months. The plants infected for the longer periods in each group had definite signs of recovery. They were given additional nitrogenous fertilizer in the hope that this would tend to correct any effects that might be due to a deficiency in nutrients. The only tests in this entire series in which some source plants showed a higher concentration of virus strain 1 for the older plants (see

TABLE 6.—Consolidated data from tests to determine the concentration of early-top virus in source plants as related to time after infection

Source and strain of virus and susceptibility of test plants	Source plant pairs ^a	Feeding periods	Total tests	Tests showing					
				No difference in virus concentration		Lower virus concentration in plants a few weeks after infection		Lower virus concentration in plant several months after infection	
				Number	Percent	Number	Percent	Number	Percent
<i>Strain 1 virus</i>									
Susceptible source plants	6	9	18	2	11	2	11	14	78
Susceptible test plants	6	7	14	0	0	2	14	12	86
Resistant test plants									
Resistant source plants									
Susceptible test plants	4	6	12	1	8	1	8	10	83
Resistant test plants	4	6	12	0	0	4	33	8	67
<i>Strain 2 virus</i>									
Susceptible source plants	4	4	10	0	0	0	0	10	100
Susceptible test plants	4	4	10	0	0	0	0	10	100
Resistant test plants									

^a Each pair of source plants consisted of two sugar-beet plants of the same variety and infected with the same early-top-virus strain, but one plant had been infected for more than 3 months and the other for a few weeks.

table 6) were among those in which the younger infections were 2 or 3 months old. This is undoubtedly explained by the fact that these plants were recovering.

Tests on susceptible plants from susceptible source plants carrying strain 1 showed a higher virus concentration for the younger infections in a very high percentage of the tests (Table 6). Of the total susceptible test plants inoculated there was 16 per cent more infection secured among those inoculated from plants which had been infected for a short time than among those which had been infected for a long time (Table 7).

TABLE 7.—*Summary of results of test-plant inoculations made during studies on age after infection as related to curly top-virus concentration in sugar beet*

Source and strain of virus and relative age of plants after infection	Resistant test plants			Susceptible test plants		
	Inoculated			Inoculated		
	Num- ber	Num- ber	Per cent	Num- ber	Num- ber	Per cent
<i>Strain 1 virus</i>						
Susceptible plants less than 3 months after infection	298	145	49	300	222	70
Susceptible plants more than 3 months after infection	297	67	23	307	165	54
<i>Strain 1 virus</i>						
Resistant plants less than 3 months after infection	251	88	35	251	181	72
Resistant plants more than 3 months after infection	254	59	23	257	134	52
<i>Strain 2 virus</i>						
Susceptible plants less than 3 months after infection	219	104	48	230	184	80
Susceptible plants more than 3 months after infection	228	38	17	230	119	52

Tests on resistant plants from susceptible source plants carrying strain 1 showed a higher virus concentration for the young infections in a still greater percentage of the trials (Table 6). Of the total resistant test plants inoculated there was 26 per cent more infection among those inoculated from plants which had been infected for a short time than among those which had been infected for a long time (Table 7).

Tests on susceptible plants from resistant source plants carrying strain 1 showed a higher virus concentration for the younger infections in a very high percentage of the tests (Table 6). Of the total susceptible test plants inoculated there was 20 per cent more infection secured among those inoculated from plants which had been infected for a short time than among those which had been infected for a long time (Table 7).

Tests on resistant plants from resistant source plants carrying strain 1 showed a higher virus concentration for the younger infections in a high percentage of the trials (Table 6). Of the total resistant test plants inoculated there was 12 per cent more infection secured among those inoculated from plants which had been infected for a short time than among those inoculated from plants which had been infected for a long time (Table 7).

Tests on susceptible plants from susceptible source plants carrying strain 2 showed a higher virus concentration for the younger infections in every case (Table 6). Of the total susceptible test plants inoculated there was 28 per cent more infection secured among those inoculated from plants which had been infected for a short time than among those inoculated from plants which had been infected for a long time (Table 7).

Tests on resistant plants from susceptible source plants carrying strain 2 showed a higher virus concentration for the younger infections in every trial (Table 6). Of the total resistant test plants inoculated there was 31 per cent more infection secured among those inoculated from plants which had been infected for a short time than among those inoculated from plants which had been infected for a long time.

It is clearly evident that, under the conditions of these experiments, the virus concentration was greater in plants a short time after infection than it was several months after infection. This was true for both strain 1 and strain 2 of the virus and regardless of whether the source plant was a resistant or a susceptible sugar beet.

DISCUSSION ²/₄

It has already been shown (3) that age of plants at time of curly-top inoculation is an important factor in the amount of infection and the degree of injury which may occur. The data here presented indicate that the relative age of infection as related to virus concentration is another factor in favor of the older beets. Both the increased resistance and the decreased virus concentration are favorable factors in any beet fields in which there are few curly-top-infected beets and a leafhopper population that does not increase for some weeks. Such conditions may well be factors of importance in connection with beets planted for seed or in other plantings made in the fall when conditions become progressively less favorable for the leafhoppers.

The presence of the more virulent curly-top-virus strain 1 in any beet field is certain to result in more rapid spread of the disease than if the virus were strain 2, as evidenced by the greater concentration of the strain 1 in either the susceptible or the resistant sugar-beet source plants (Tables 4 and 5). Although other curly-top-virus strains producing relatively serious or slight injury to sugar beet have not been studied it is reasonable to expect that their comparative concentrations in infected sugar-beet plants would vary in a manner similar to those of strains 1 and 2.

The data in table 3 show that, under the conditions of these experiments, the percentage of infection secured from resistant source plants carrying strain 1 was only 23 when resistant test plants were used while the same virus from susceptible source plants gave 65 per cent infection among susceptible test plants. This would indicate that the rate of spread of strain 1 curly-top virus in a field of susceptible sugar beets might well be approximately three times as rapid as it would be in a field of the highly resistant beets, assuming that each field had the same amount of initial infection and

the same population and distribution of the vector. In the case of strain 2 virus there was only 12 per cent infection secured from resistant source plants when resistant test plants were used, while the same virus from susceptible source plants gave 72 per cent infection among susceptible test plants. This would suggest the possibility for six times as rapid a rate of spread of virus strain 2 among the plants in a field of susceptible beets as in a field of resistant beets.

Field conditions would rarely, if ever, be closely similar to those used in these experiments, but the general principles which brought about these results in the greenhouse would certainly be important factors in commercial beet fields. In other words, it is clear that the rate of spread is certain to be greatly reduced in fields of resistant beets and that the presence of susceptible beets adjacent to the resistant fields would greatly increase the rate of spread. Resistant sugar-beet varieties are well known for their ability to resist severe injury when infected and for the lower rate of infection that results when they are inoculated. The much lower virus concentration carried by such resistant beets is another important factor in their favor.

SUMMARY

The curly-top-virus concentration is much greater in infected susceptible sugar beets than in infected resistant sugar beets. This is true for both strain 1 which is highly virulent and strain 2 which is relatively low in virulence. The difference in virus concentration between resistant and susceptible plants is much greater in those infected with strain 2 than in those infected with strain 1.

The curly-top-virus concentration is much greater in sugar-beet plants infected with the highly virulent strain 1 than in those infected with the less virulent strain 2. This was true in both the susceptible sugar beet and the resistant sugar beet. The difference between strains 1 and 2 in virus concentration is much greater in resistant source plants than in susceptible source plants.

The curly-top-virus concentration is much greater in sugar-beet plants three weeks to twelve weeks after infection than in plants which have been infected for three to eight months. This is true when the source plants are infected with either virus strain 1 or with virus strain 2 and, in the case of strain 1, whether the source plant is of the resistant variety or the susceptible variety.

The χ^2 test shows high significance in the virus concentration differences found between infected plants of the resistant sugar-beet variety S.L. 68 and the susceptible variety S.L. 842; between plants infected by strain 1 virus and those infected by strain 2 virus; and between those which have been infected for 3 to 12 weeks and those which have been infected for 3 to 8 months.

The lower virus concentration in resistant sugar beets is a favorable

factor, reducing the rate of spread of curly-top infection among such varieties.

In all groups which were studied the resistant sugar-beet test plants gave more striking evidence of differences in virus concentration than the susceptible test plants. This indicates the possibility of mass action as a factor related to infection (4).

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MASS ACTION AS A FACTOR IN CURLY-TOP-VIRUS INFECTION OF SUGAR BEET

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INTRODUCTION

The term "mass action," as used in this article, is defined as the effect of the varying concentrations of the reacting masses on infection whether the reactions involved are reversible or not.

Carsner and Lackey (3) stated that the relation of mass action to curly-top infection has been shown: "(1) by varying the amount of inoculated virus by (a) inoculations with contrasting numbers of leafhoppers and (b) unequal periods of exposure; (2) by use of plants differing in susceptibility; (3) by studies on the incubation period of the virus in the insect; and (4) by comparing the minimal infective doses of the virus in its virulent and attenuated condition." They did not publish the data upon which these conclusions were based.

Severin (10) says "The relation of mass inoculation by groups of beet leafhoppers to curly-top transmission was demonstrated by varying the time of exposure of the insects on healthy beets." Study of his data suggests the possibility that mass action might be an infection factor but the evidence is not convincing.

There are many important variables which require consideration in the study of mass action as related to curly-top infection. Extreme variation among individual leafhoppers as to efficiency in transmitting the curly-top virus, *Ruga verrucosa* Carsner and Bennett, has been reported by Carsner and Stahl (4), Severin (10), Freitag (5), and Bennett and Wallace (2).

The use of different curly-top-virus strains or of mixtures of strains might readily account for extreme variation in results. It is certain that no consideration was given to strains in the earlier work, since their existence was not known at that time.

Comparisons between the amounts of infection produced by leafhoppers that had been fed on two different diseased plants might easily be misleading unless differences in virus strains or virus concentrations in those diseased plants are considered (9). Giddings (7) has reported that leafhoppers fed for a short time on diseased plants containing virus strain mixtures showed extreme irregularity in the strains that they transmitted and this is another source of variability.

While the conclusions reached in this paper are in general agreement with those of the earlier workers it is believed that the evidence presented herewith is such as to give those conclusions a far more substantial foundation.

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The work reported in this paper was not undertaken for the purpose of learning the possible rôle of mass action as related to curly-top infection of sugar beets, and the data submitted are by-products of experiments dealing with virus concentration (9). A tabulation of results from a group of curly-top-virus-concentration experiments was found to furnish such strong evidence of mass action as a factor in infection that all such experiments were checked for similar evidence.

METHODS

The data were secured from sugar-beet plants of the highly resistant variety S.L. 68 and the very susceptible variety S.L. 842. The curly-top-virus strains involved were those previously described (6) as strain 1 and strain 2. The methods of feeding leafhoppers on source plants and of inoculating and handling the test plants are described in detail elsewhere (9). The periods of feeding on source plants were 30 minutes, 1 hour, and 2, 3, 6, 9, 15, and 24 hours. Each leafhopper then was allowed to feed for a week upon the cotyledon of one test plant.

If the experiments had been set up for the study of mass action the lengths of feeding period on infected (source) plants might have been somewhat different, the numbers of plants and leafhoppers involved would have been kept closely similar for each feeding period and some other variables would have been avoided. Since the data available appeared entirely adequate to prove the importance of mass action, it was deemed unwise to conduct further experiments merely in an effort to present a clear picture for each of the feeding periods mentioned. Therefore, the data from 30-minute feedings were combined with those from 1 hour, those from 2 hours with those from 3 hours, and those from 6 hours with those from 9 hours. In each case the longer period of feeding involved a much larger number of plants.

EXPERIMENTAL RESULTS

It was well known from earlier work (6) that the percentage of infection secured among inoculated beets of the resistant variety by either virus strain 1 or strain 2 was normally less than the percentage of infection among plants of susceptible varieties. This is further indicated by the results given in table 1 for controls; that is, inoculations with leafhoppers reared on source plants. If such a difference in amount of infection secured under controlled conditions were due to some factor other than mass action then the relative percentages of plants infected should remain proportional when the amount of inoculum carried by the leafhopper is greatly reduced. It is evident from the data in table 1 that such is not the case. The ratio of infections was 1:1.1 among resistant and among susceptible plants when using single leafhoppers that had been reared on infected plants, whereas the ratio was 1:2.9 for those leafhoppers that had been fed for one hour or less on an infected plant. The data for each of the successively shorter feeding periods show a striking consistency in the change of ratio.

The relation between length of feeding time on source plant and percentage of infection among inoculated plants (Table 1, columns 4 and 7) is also believed to be significant. The low percentage of infection secured from the short feeding periods might be partially explained by the possibility that some of the leafhoppers had failed to feed on the source plant, or had obtained only water from it during the 1-hour or 3-hour feeding periods but this could hardly be an important factor in the longer feeding periods. Bennett (1) has shown that very few leafhoppers survive more than two or three days at room temperatures if feeding is limited to parenchyma tissues or tap water, so it is reasonably certain that an appreciable quantity of the curly-top virus

TABLE 1.—*Evidence of mass action as indicated by differences in extent of infection of resistant and susceptible test plants in relation to period of feeding of vector on curly-top virus source plants^a*

Feeding period on virus source plant	Resistant test plants			Susceptible test plants			Ratio of infected resistant to infected susceptible
	Inocu- lated	Infected	Infected	Inocu- lated	Infected	Infected	
	<i>Number</i>	<i>Number</i>	<i>Per cent</i>	<i>Number</i>	<i>Number</i>	<i>Per cent</i>	
1 hour ^b	1703	109	6.4	1714	317	18.4	1: 2.9
3 hours ^c	1994	431	21.6	2036	1063	52.2	1: 2.4
9 hours ^d	624	212	34.0	631	444	70.4	1: 2.1
15 hours	227	89	39.2	232	159	68.5	1: 1.7
24 hours	231	121	52.0	223	168	75.3	1: 1.4
Controls (reared on source plant)	1538	1228	79.8	1566	1433	91.5	1: 1.1

^a The data in this table were submitted to the χ^2 test which indicated highly significant differences between the infection percentages.

^b Combined data for 30 minutes and 1 hour.

^c Combined data for 2 hours and 3 hours.

^d Combined data for 6 hours and 9 hours.

was picked up by every leafhopper which fed 15 or 24 hours upon the source plant. During one week of feeding upon the test plant it seems highly probable that such a leafhopper vector would inject at least a few virus particles into this plant and that the plant would become infected if a small number of virus particles could induce infection. Both susceptible and resistant test plants show successively large increases in the percentage of plants infected by leafhoppers fed for these longer periods and for the controls (Table 1), indicating that mass action must be a factor.

DISCUSSION AND SUMMARY

The data presented in this paper show very strong evidence that mass action is an important factor in the curly-top infection of either the resistant sugar-beet variety S.L. 68 or the susceptible variety S.L. 842. The reactions involved are initiated by the introduction of the curly-top virus into the plant. If the active mass of the virus introduced into the plant is sufficient to induce predominant reactions the final result is multiplication of the virus

and consequent infection of the plant. A smaller active mass of virus initiates the same reactions as the larger active mass but, because of the inadequate amount, the degree of the reaction is so limited that the final result does not permit multiplication of the virus. According to this hypothesis, the amount of active mass of virus required to bring about infection would vary with different beet plants and especially with different beet varieties because of the differences in the active mass of the specific reacting substances in the plant. It would appear probable that the reactions involved in the case of curly-tip virus are similar to those which occur in many bacterial and fungus infections in which mass action is a factor.

If mass action is a factor in the case of beet varieties S.L. 68 and S.L. 842, it is undoubtedly a factor for other beet varieties and for other plant species.

Evidence from field experiments on age of plants as a factor in resistance to curly top (8) gives support to the mass action concept. It would seem that, as the plants become older, there is an increase in them of the active mass with which the virus active mass must react before virus multiplication may take place.

The evidence that mass action is an important factor in infection should give added stimulus to the effort to reduce the amount of curly-top virus available in the vicinity of commercial fields of sugar beets or of other crops which suffer from the curly-top disease. The growing of curly-top-resistant beets and the elimination of weed plants which serve as virus reservoirs can be of great value in decreasing the amount of virus available to the insect vector. This would result in a smaller relative mass of inoculum and a consequent less amount of curly-top infection of beets and other crops in areas where this disease is prevalent.

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STRIPE SMUT (*USTILAGO STRIAEFORMIS*), IN RELATION TO BLUEGRASS IMPROVEMENT¹

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In 1940 the West Virginia Agricultural Experiment Station, in cooperation with the U. S. Regional Pasture Research Laboratory, undertook a program of bluegrass (*Poa pratensis* L.) improvement as part of a broader program of pasture improvement. Among the objectives of the project were studies of variation within the species, the selection of types best adapted to specific systems of management, and the breeding of strains with desirable characters, including resistance to disease. Preliminary studies (10) already reported have shown that there was much variation among individual-plant selections of bluegrass when they were grown in a nursery for observation. Mildew, rust, smut, and several fungus leaf spots were prevalent in the nursery, and differences in resistance of individual plants to the various diseases were obvious. In the nursery where the grass was not kept clipped some of these diseases appeared to be more prevalent than they are in most pastures. Further observation indicated that this was true for mildew, rust, and most leaf spots, but not for smut. Comparisons made of the prevalence of smut in the nursery and in pastures showed a close correlation between its presence in the nursery collections and its presence in the pastures from which the nursery collections were made.

The greater prevalence of rust, mildew, and leaf spots in the nursery as compared to that in pastures can be explained by the greater opportunity for building up inoculum on the unclipped grass of the nursery than on the grazed grass of the pastures. Because of the systemic nature of smut infection, its development and spread are less dependent on unrestricted growth of the plant than are rust, mildew, and the leaf spots. On the other hand there is some reason to believe that development of smut and its spread into new tillers may be stimulated by clipping or grazing. These facts all lead to the conclusion that smut is potentially the most destructive disease of bluegrass in pastures.

Pasture surveys made in West Virginia and in Pennsylvania have borne out this conclusion (10). No pastures were found to be entirely free from smut, and by actual count of representative quadrats several pastures were found in which more than 25 per cent of all bluegrass plants were affected. Large areas with more than 50 per cent infection are not uncommon. In dry weather many of these plants die, resulting in poor pastures that otherwise would be quite productive. It is obvious that smut resistance must be given prime consideration in any program of bluegrass improvement. It is probable that in the mixed population found in a pasture many individual plants

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are resistant, while others are very susceptible. Should a small number of apomictic lines be selected without regard to smut resistance and be introduced as improved strains, they might easily prove worthless in the presence of smut infection.

Because of the prevalence and destructive nature of smut in present-day pastures and its still greater potential importance, a study of the disease was undertaken with particular reference to the problem of testing strains of bluegrass for smut resistance. It is a recognized principle that in breeding for resistance to a disease a technique of producing infection approaching 100 per cent on susceptible varieties is necessary for satisfactory elimination of susceptible strains. Since bluegrass smut had not been extensively investigated from this viewpoint no satisfactory technique was available, although some inoculation experiments had been reported.

LITERATURE REVIEW

Ustilago striacformis (West.) Niessl is recognized as a composite species affecting many grasses. There is abundant evidence (3, 4, 5) that it is physiologically specialized on the various grass hosts, although the exact host range of each physiologic form has never been determined. Davis (4) recognized four physiologic forms (or races) as follows: forma *Phlei* on *Phleum pratense* L.; forma *Agrostidis* on *Agrostis palustris* Huds.; forma *Poa-pratensis* on *Poa pratensis* L., and forma *Poa-annuae* on *Poa annua* L. The stripe smut on orchard grass was given specific rank and named *Ustilago clintoniana*. Fischer (5) studied a race of the smut affecting species of *Agropyron*, *Elymus*, *Hordcum*, and *Sitanion* and designated it as forma *Hordei*.

While these different physiologic races of the smut are morphologically and symptomatically similar they appear to differ not only in their parasitic specificity, but also in methods of chlamydospore germination, methods of infection, and in cultural characteristics. Davis (2, 4) working with spores from smutted timothy, red top, bluegrass, and orchard grass concluded that they would not germinate until they had passed through a resting period of about 240 days. He stated that the type of germination was the same for spores from all four host plants. "The promycelia were at first unicellular and multinucleate, but under certain conditions became multicellular with four to five lateral sporidia; however, occasionally only one lateral sporidium formed on the sides. The granular protoplasm assembled in the tips of unicellular promycelia, which sometimes formed lateral sporidia. Secondary spores or buds were usually formed from conidia, primary sporidia, tips of promycelia and other buds. . . . Lateral sporidia sometimes fused and formed conidia which developed buds and mycelial threads." Davis was unable to culture the fungus in decoctions, agars, and other media.

Fischer (5), on the other hand, found no after-ripening period necessary for germination of chlamydospores of forma *Hordei*. The process of germination also differed from that described by Davis. Two or three thick germ

tubes emerged from each spore. These developed into branched septate promycelia from which typical elliptical sporidia were budded in profusion. The sporidia grew and developed into large sporidial colonies on nutrient agar. This was the first record of the cultivation of *Ustilago striaeformis* on artificial media. The sporidia were found to be unisexual and fusions occurred when sporidia of opposite sex were mixed together on nutrient agar. Long, vigorous aerial infection hyphae arose from each fused pair of sporidia.

Kreitlow (6), working with forma *Agrostidis*, found that fresh chlamydospores usually did not germinate, although one collection of spores germinated without a rest period. Later (7) he was able to break the rest period of spores from *Poa pratensis* by subjecting them to a temperature of 35° C. in a moist chamber for 25 or 30 days. Spores of forma *Agrostidis*, according to Kreitlow, germinated by forming a single branched or unbranched promycelium 20 to 50 microns in length. No sporidia were observed, although lateral branches resembling sporidia were observed on some rapidly growing promycelia. The method of germination of the chlamydospores from bluegrass was not described.

Kreitlow (6) found that germinating chlamydospores of forma *Agrostidis*, when transferred to agar, grew readily, but formed strictly mycelial colonies in contrast to the sporidial growth obtained by Fischer (5) with forma *Hordci*.

Davis (2, 4), after extensive inoculation experiments with the smut on several different hosts, concluded that infection occurred in the seedling stage, penetration taking place through the coleoptile. Efforts to inoculate other tissues of growing plants were unsuccessful. Floral infection was not obtained and there was no evidence that the smut was seed borne. Fischer (5), on the other hand, found that seed taken from smut-infected plants of slender wheat grass produced only smutted plants, indicating that, on this host at least, the fungus is seed-borne.

Such wide variation in the behavior of the smut, as reported on various hosts by different investigators, would make it unwise to generalize too much about the life history of *Ustilago striaeformis*. Since the smut has been studied less extensively on bluegrass than on almost any other host plant, a thorough investigation of its life history on this host seemed necessary as a basis for working out any practical method of artificial inoculation.

EXPERIMENTAL RESULTS

Chlamydospore Germination and Culture on Artificial Media

The work reported here deals primarily with a study of the fungus in artificial culture and with inoculation experiments in the greenhouse. Abstracts of certain aspects of the work have been published (8, 9).

Life-history studies were started with attempts to germinate the chlamydospores. Spores from infected plants growing in the nursery were collected in late summer and attempts were made to germinate them in distilled

water and various nutrient solutions, as well as in and on the surface of plain and nutrient agar. Very few spores (never as much as 1 per cent) germi-

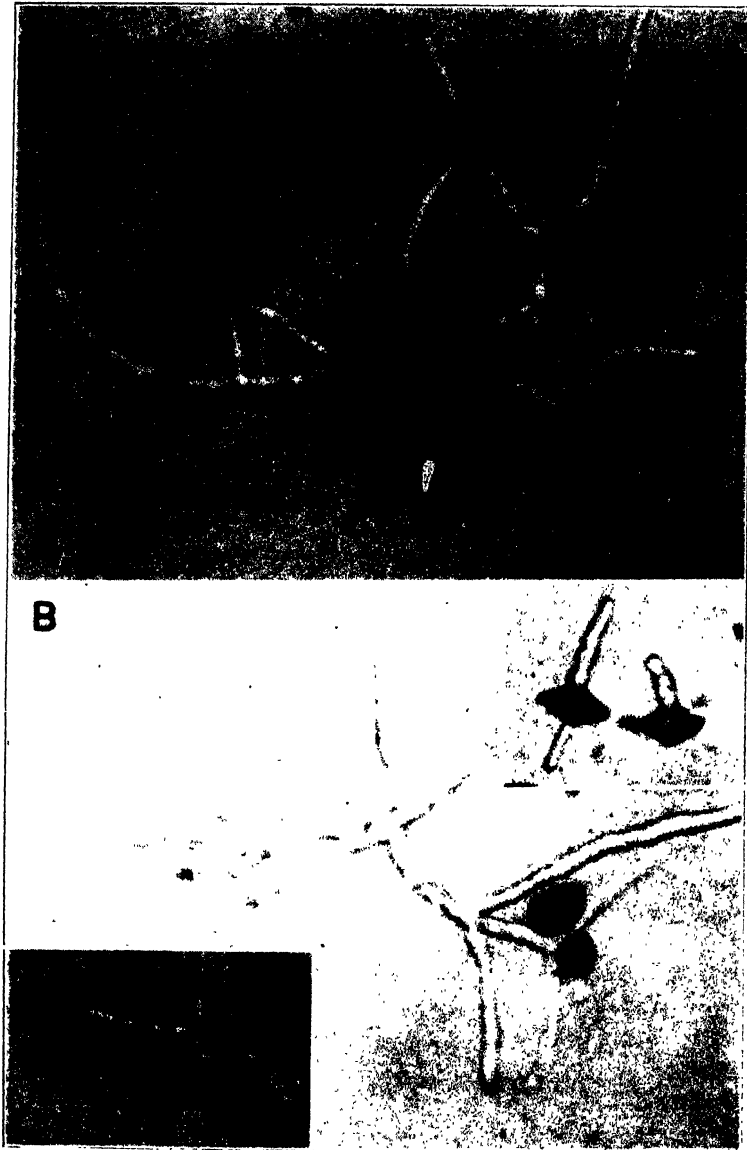


FIG. 1. Chlamydozoospores of *U. striatiformis* germinating in 2 per cent malt extract. The promycelium is indeterminate, the germ tube is branched, and sporidia are lacking. $\times 640$. A, spores from bluegrass; B, spores produced in artificial culture.

nated under any of the conditions provided and there were no significant differences between the amounts of germination on the various media used. On the assumption that failure of the spores to germinate was caused by an

acquired dormancy, attempts were made to germinate young spores taken from unruptured pustules. This was done on the theory that dormancy might be induced by some change in the permeability of the spore wall after exposure to the air. Fresh leaves showing early stages of infection were taken from greenhouse plants and surface sterilized in a mercuric chloride solution, (1-1000), and then washed in sterile distilled water. Sections of the leaf were cut aseptically through a young unruptured smut pustule. These leaf sections were placed in the bottom of a sterile Petri dish in a drop of sterile distilled water and cut into numerous small segments with a sterile scalpel. A tube of melted agar was then poured over the segments and the plate incubated at room temperature. Microscopic examination showed that numerous chlamydospores had been released from the pustules and were distributed throughout the agar. These spores did not germinate much better than those used in previous studies, but the few that did germinate grew in the agar and could be picked up aseptically and transferred to tubes of agar for further growth.

The chlamydospores germinated by forming one, or occasionally two or three, simple or branched germ tubes with no sporidia. In distilled water the germ tubes are usually unbranched or sparsely branched, but in agar or in nutrient solutions the tubes branched profusely (Fig. 1, A) and grew into characteristic colonies. Typical promycelia and sporidia have not been observed.

Even though only a few chlamydospores germinated, numerous small fungus colonies appeared in the agar near and adjacent to the smut pustules (Fig. 2, C). Since these resembled the colonies arising from germinating chlamydospores they were objects of interest. Further study showed that these colonies arose from fragments of smut mycelium from the leaf tissue adjacent to smut sori. Numerous colonies were picked out of the agar and subcultured on agar where they grew slowly into small white bead-like colonies when crowded (Fig. 3, E) or into larger colonies with a characteristic convolute surface when isolated (Fig. 3, A). When the colonies were examined microscopically most of them were found to be composed of a mycelium that broke up readily into short, sporidia-like fragments. These sporidia-like bodies, unlike most smut sporidia, do not multiply by budding; but each fragment, by apical growth, may form more fragmenting mycelium (Fig. 2, D, E). On subculturing these fragments an occasional, typical, mycelial colony appeared. The mycelial colonies formed a tough, velvety mat and the hyphae showed no signs of fragmentation. The two types of colony could usually be detected in very early stages of growth by microscopic examination. The young fragmenting colonies tended to be compact and the individual hyphae were characteristically curved and twisted, while the mycelial colonies were more spreading with straight hyphal branches (Fig. 2, A, B). Older colonies of the two types are shown in figure 3, A-D.

On subculturing the two types of colonies there was a tendency of the fragmenting colonies to dissociate and produce the typical mycelial colonies,

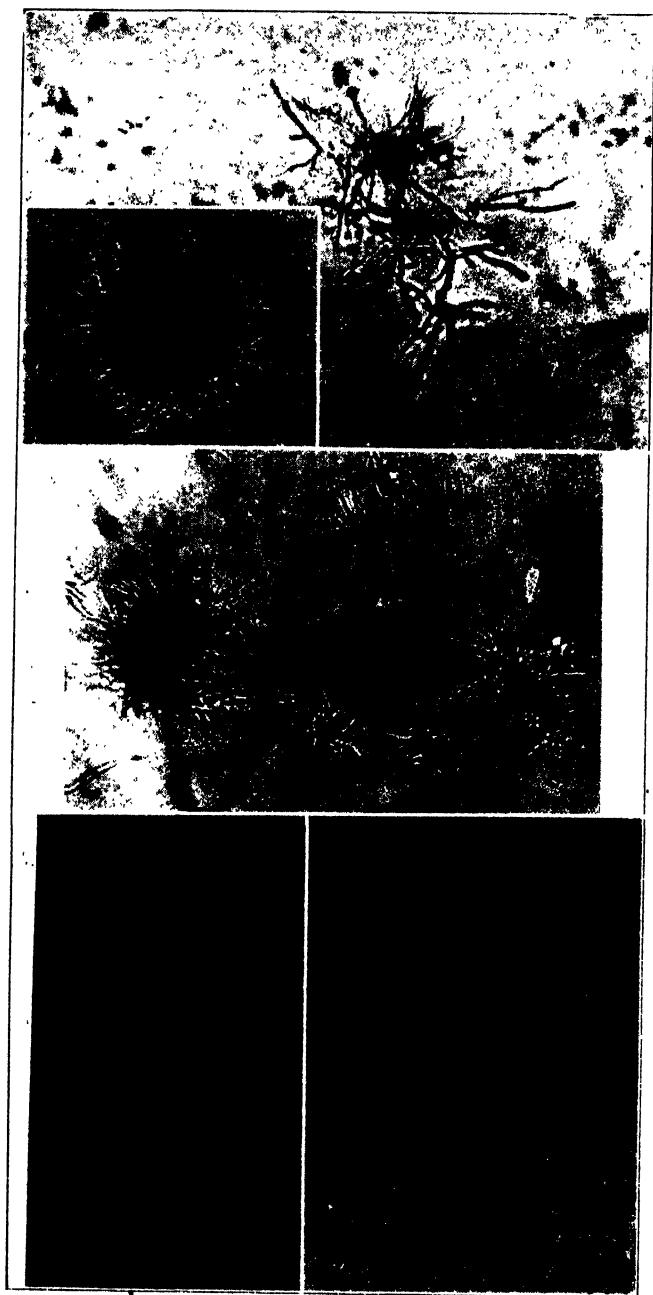


FIG. 2. Young colonies of *U. striiformis*: A, fragmenting type; B, mycelial type; C, fragmenting type originating from mycelial fragments freed from immature pustules of smut plated on potato-dextrose agar. D and E, mycelial fragments of which a fragmenting colony is composed. A through D, $\times 125$. E, $\times 560$.

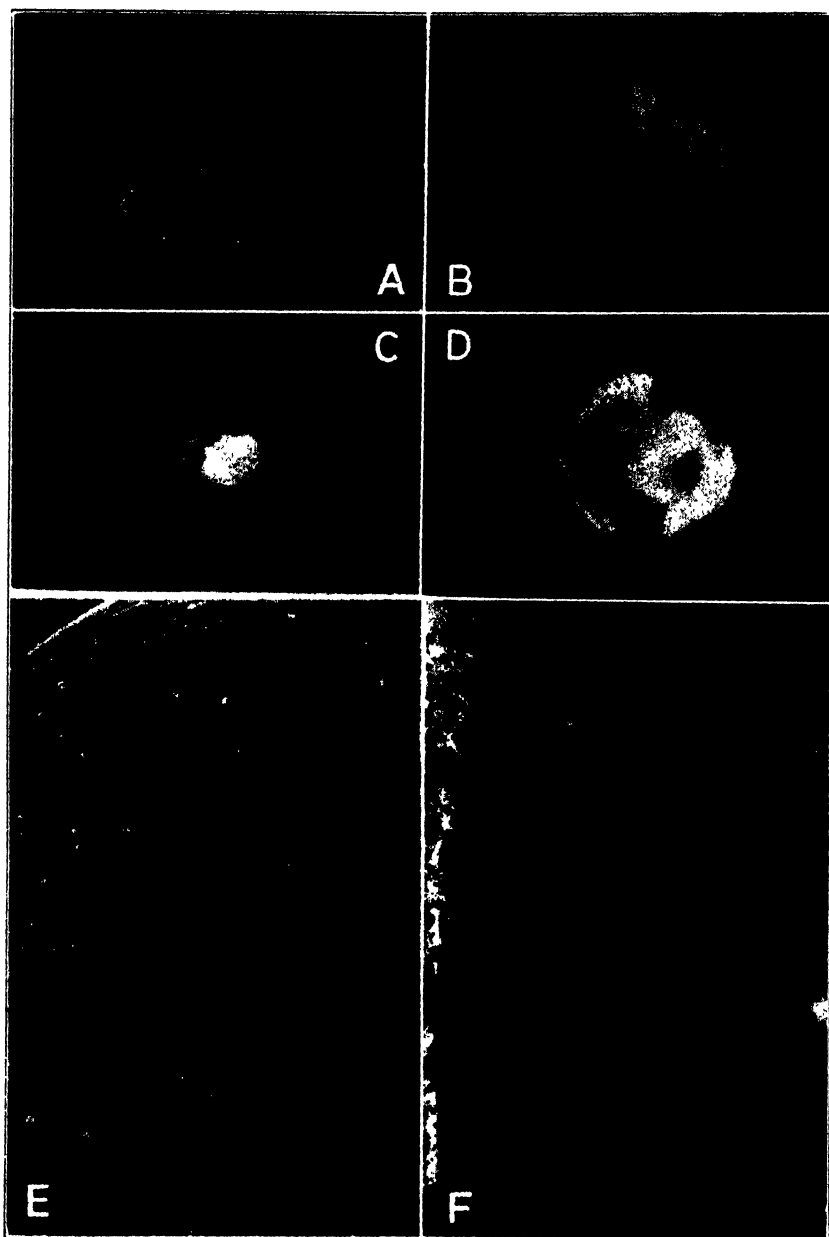


FIG. 3. Macroscopic view of colonies of *U. striaeformis*. A, fragmenting type in which the mycelium breaks up into short sporidia-like fragments. B, mycelial type in which there is no fragmentation of mycelium. C, a fragmenting colony that has produced a mycelial sector. D, a mycelial colony beginning to form chlamydospores in localized areas. A through D, giant colonies, natural size. E, small white bead-like fragmenting colonies arising from mycelial fragments isolated from immature smut pustules. Natural size. F, black masses of chlamydospores formed on the surface of an agar plate seeded with mycelial fragments from a fragmenting colony. The culture in early stages formed white bead-like fragmenting colonies as in E. In spore formation practically all of the mycelial fragments are transformed into chlamydospores. Slightly enlarged.

either as sectors (Fig. 3, C) or as individual colonies from separation plates. Both types of colony grew very slowly when first isolated, but after several subcultures there was an appreciable increase in the rate of growth as if the fungus had adapted itself to growing in artificial media.

After several subcultures on potato-dextrose agar some of the cultures of the fragmenting type changed from the usual glistening white to intense black (Fig. 3, F). On microscopic examination it was found that chlamydospores were being produced in abundance. The spores were similar to those formed on the host plant, but some of them were not typical in all respects. Chlamydospores produced in culture tended to be slightly larger than those produced on the host plant and the echinulations, although present, were not so clearly defined. Also there was usually a number of abnormally shaped spores; many of them tended to be more oblong than normal and many were lemon-shaped with characteristic pointed ends (Fig. 4).

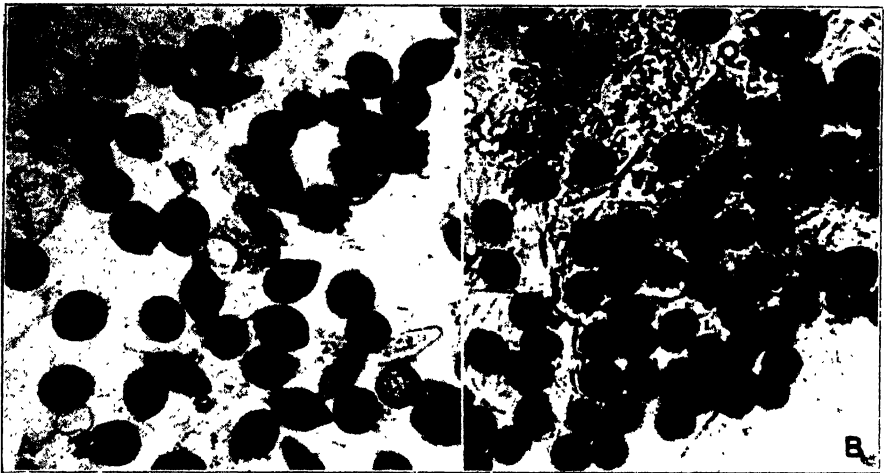


FIG. 4. Chlamydospores of *U. striariformis*: A, produced on potato-dextrose agar; B, produced on bluegrass. $\times 500$.

Sporulation at first occurred only in fragmenting colonies, but later certain typical mycelial colonies began to form chlamydospores (Fig. 3, D). Also certain isolates tended to form spores, while others seemed unable to do so. However, many of the apparent "nonsporulating" cultures suddenly began to sporulate after numerous subcultures had failed to form spores.

At first it appeared that the chlamydospores formed in culture would not germinate, but while the work was in progress Kreitlow (7) reported his success in inducing germination by subjecting the chlamydospores to a temperature of 35° C. in a moist chamber. When this method was tried on chlamydospores produced in culture they germinated as well as those produced on the host plant, and no difference in the methods of germination was observed (Fig. 1, B). Later some lots of spores from culture germinated fairly well without treatment, ranging from 5 to 50 per cent in different trials.

The significance of the various types of growth on artificial media has not been fully determined. Cytological studies are under way and the results will be published in another paper.

Inoculation Experiments

While the fungus was being studied in culture, inoculation experiments were being made in the greenhouse. Inasmuch as the experience of other workers had indicated seedling infection to be the general rule for this smut, seed inoculation was the first method tried. Fresh chlamydospores obtained from the field in late summer were used. At this time no means of inducing a high percentage of spore germination was known and the spores used germinated less than 1 per cent in distilled water. Several methods of seed inoculation were tried. One lot of seed was dusted with dry spores immediately before planting, another lot was soaked for two hours in a suspension of spores before planting. A third lot was soaked for two hours in a spore suspension during which it was subjected periodically to a moderate vacuum.

TABLE 1.—*Results of seed inoculation of bluegrass with fresh chlamydospores of Ustilago striaeformis, obtained from infected grass*

Method of inoculation	Percentage of infection
Dry spores dusted on seeds	2
Seeds soaked 2 hr. in spore suspension	0
Seeds soaked 2 hr. in spore suspension with vacuum treatment	3
Dry seed, no inoculation	0

Several hundred seeds of each lot were planted in rows in greenhouse flats. No infection was observed after six weeks' growth, at which time 100 plants of each treatment were transplanted into separate flats for further observation. The plants remained in these flats for about 5 months during which time a few of them became infected. The plants were then transplanted into the field for further observation, but the percentage of infection did not increase. The results of one representative set of seed inoculations are given in table 1. This method of inoculation was not very effective and, unless it can be greatly improved, it would be of no practical value in the selection of resistant types. The low percentage of infection obtained was assumed to be due to the failure of a sufficient number of chlamydospores to germinate at the proper time for infection.

Some time later, after the fungus had been isolated in pure culture on agar, a similar experiment was made comparing fresh chlamydospores from grass with inoculum grown on agar. The latter inoculum was suspended in water in a small flask with glass beads. By shaking the flask the clumps of spores or mycelium were separated into a fairly uniform suspension. A similar suspension of spores was prepared from smutted bluegrass leaves. The two suspensions were then transferred to the grass seeds in small vials in duplicate. One set of seeds was allowed to soak for two hours and then

the suspension was poured off and the seeds planted. The other set was placed in a vacuum for two hours during which time the vacuum was released momentarily four times. Two lots of seeds were given the same treatment without inoculum and used as checks. The eight lots of seeds were then planted in flats of greenhouse soil. One month after planting, 100 plants from each treatment were transplanted into flats where they were kept under observation for six months. The results were almost entirely negative, only one of the 600 inoculated plants becoming infected, this being one from the lot of seeds inoculated with mycelium fragments from an agar culture and the seed soaked 2 hours with no vacuum.

With the poor results obtained by seed inoculation it was necessary to try other methods. Inasmuch as chlamydospores formed in nature probably lie in the soil some time before germination it was decided to try soil inoculation.

On July 2 six 10-inch pots of soil were prepared. Two pots were thoroughly drenched with a suspension of fresh smut spores. Two pots were drenched with a suspension of spores and mycelial fragments from a spore-forming culture of smut grown on agar. The remaining two pots were not inoculated and were retained as checks. One pot of each pair was planted immediately with bluegrass seed. All pots were imbedded in the soil in an outdoor cold frame where they remained until October 4 when they were returned to the greenhouse. At this time the second pot of each pair was planted with bluegrass.

On October 8 the plants of the early planting were examined for infection and all plants showing no infection were then transplanted to flats of clean soil for further observation. They were examined at frequent intervals until the following June when the experiment was discontinued. Whenever an infected plant was found it was recorded and removed from the pot. Records were kept on the second planting until the following September. The results of this experiment are given in table 2.

It is evident from the results of these experiments that a much higher percentage of infection was obtained by soil inoculation than by seed inoculation. The most infection was obtained when chlamydospores from infected plants were used as inoculum. Inoculum grown on agar was much less effective, although some infection was obtained. It should be noted that infection was very slow in developing and that it required over 300 days for infection to show on some plants. It is not known whether this long time is due to a long incubation period within the plant, or whether the fungus persisted in the soil as mycelium or viable chlamydospores and infected the new tillers formed as the plants grew. Since the plants were transplanted into clean soil after 98 days it might be assumed that they were infected before transplanting, but as some soil was transferred with the plants some inoculum also could have been transferred.

In an effort to determine whether the fungus would persist in the soil and be capable of causing infection for an extended period, pot No. 1 was replanted without further inoculation on October 8 when the original plants

were transplanted. The new plants were allowed to grow until March 10 of the following year when they were removed and those showing no infection were transplanted into flats for further observation. On March 14 the pot was reseeded for the third time. The plants of the third crop were allowed to grow until July 22 when they were removed.

The infection occurring on these plants seeded into the previously inoculated soil was as follows: the first replant, or the second crop of plants, seeded 98 days after inoculation of the soil, produced 17 infected plants out of a total of 71 plants, or 23.9 per cent after 229 days. The second replant seeded

TABLE 2.—*The results of soil inoculation with two kinds of inoculum of U. striaeformis on bluegrass*

Pot No.	Inoculum and inoculation procedure	Percentage of infection	Days after planting
1	Fresh spores from infected plants; seeded immediately	25.30	98
		54.80	217
		69.90	250
		77.40	272
		87.00	327
2	Fresh spores from infected plant; seeded 3 months after soil inoculation	5.70	123
		27.00	155
		33.30	169
		52.00	208
		60.00	234
		62.80	246
3	Spores and mycelium from agar culture; seeded immediately	5 or 6 plants out of nearly 200 plants—exact percentage not determined	98
		No increase	280
4	Spores and mycelium from agar culture; seeded 3 months after soil inoculation	3.00	126
		9.10	172
		9.10	290
5	Check, no inoculum; planted immediately	0.00	240
6	Check, no inoculum; planted 3 months after soil inoculation	0.65	132
		1 plant out of 152	280
		0.65	280

256 days after inoculation of the soil yielded 7 smutted plants out of 355 plants, or approximately 2 per cent after 100 days.

It is evident from these results that the inoculum does persist in some form in the soil under greenhouse conditions for as long as 256 days, although it decreases materially during this period. It is not possible to determine from this experiment whether the extremely long time required for the appearance of smut on some of the plants was due to the prolonged incubation period or whether infection occurred on older plants from soil-borne inoculum. Further experiments designed to answer this question are under way.

Working with *Ustilago striaeformis* on timothy, Davis (2, 4) concluded that seedlings were most susceptible when coleoptiles were 1 to 10 millimeters long. No infection was obtained by Davis after the coleoptiles were more than 16 millimeters long. The smut of bluegrass apparently is not

limited to such a short period of infection. Evidence that infection takes place on older seedlings of bluegrass was obtained as a by-product of an experiment designed to compare the effectiveness of different kinds of inoculum.

Seven glass culture dishes of moist sandy soil were steam-sterilized. On February 14, 1944, one dish was inoculated with a suspension of chlamydo-spores from bluegrass. A second dish was inoculated with mycelial fragments from a nonsporeforming culture grown on agar. A third dish was inoculated with a suspension of chlamydo-spores from an agar culture. One week later a second set of dishes was inoculated in the same way and all six dishes, together with an uninoculated check, were planted with bluegrass seed. Three weeks after planting 100 seedlings from each dish were transplanted to flats of soil where they were allowed to grow for six months, after which the experiment was discontinued. It is not necessary to tabulate the results of this experiment for only two of the 700 transplanted plants became infected. These were from the dish inoculated with spores from bluegrass one week before seeding. This experiment was planned on the assumption that, like the smut on timothy in the experiments of Davis (2, 4), infection would occur on the very young coleoptiles. As a matter of curiosity one dish, namely the one inoculated with spores from grass at time of planting, was saved and the plants were kept growing in the inoculated soil. On May 31, a little more than 3 months after planting, the plants were examined and 15 out of 337 were found infected. The 322 remaining plants were transplanted to flats and kept under observation until January 12, 1945, during which time a total of 78 or 23 per cent became infected. These results would indicate that the plants in the experiment were removed from the inoculated soil too early and that most of the infection occurred on plants more than three weeks old.

With this evidence that infection is not confined to very young seedlings, it was decided to try several other methods of inoculation. As a preliminary experiment twenty bluegrass plants growing in clean soil were selected, each of which had one or more young developing tillers. These were washed and the roots and tillers were dipped momentarily in a suspension of chlamydo-spores from bluegrass, after which they were transplanted into 4-inch pots of clean soil and frequently observed, so that infected plants were observed as soon as symptoms appeared. The records of this inoculation experiment are as follows:

105 days after inoculation,	2 plants	infected = 10 per cent
109 do	3 do	= 15 per cent
121 do	4 do	= 20 per cent
161 do	5 do	= 25 per cent
191 do	8 do	= 40 per cent
195 do	11 do	= 55 per cent
227 do	12 do	= 60 per cent

These results would indicate that infection is not confined to very young seedlings and that parts of older plants, probably young tillers, may become infected readily.

If infection is not limited to young seedlings it seemed possible that inoculum injected into growing plants at or near the growing point might prove effective. Accordingly, 25 vigorously growing seedlings were inoculated by injecting a suspension of fresh chlamydospores through the sheaths at the base of the stem near the growing point. This operation was per-

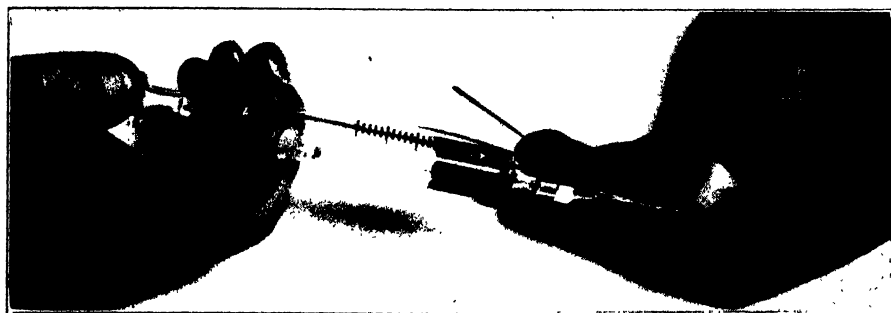


FIG. 5. The method used in inoculating bluegrass plants by injecting, with a hypodermic needle, a suspension of smut spores. An effort was made to place the spores as near as possible to the growing point without seriously injuring the plant.

formed by removing the plant from the soil and injecting the spore suspension with a hypodermic needle as shown in figure 5. The plants were then transplanted into flats or pots of soil and watered thoroughly. Infection was obtained as follows:

90 days after inoculation,	6 plants infected = 24 per cent
123 do	9 do = 36 per cent
145 do	10 do = 40 per cent
242 do	12 do = 48 per cent

This experiment showed that infection occurs when chlamydospores are injected with a hypodermic syringe into the stem of bluegrass plants. Although the avenue of infection is not known definitely, it is probable that the young leaf tissues at or near the growing point constitute the infection court.

When it was found that a relatively high percentage of infection could be obtained by injecting spores into the stems, similar inoculations were made using mycelium and chlamydospores from agar cultures, but very poor results were obtained. At the time these experiments were made all attempts to germinate the spores produced in agar cultures had failed.

In the meantime inoculation experiments have been devoted to improving the effectiveness of the hypodermic-needle method. This method has much promise in the development of a practical means of eliminating susceptible strains of bluegrass and identifying the resistant ones. If we were dependent upon seed inoculation, resistant strains could not be identified without some method of inoculation that would give practically 100 per cent infection on susceptible types. Otherwise, escapes could not be tested until seeds were produced and results of second generation tests would be complicated by genetic variations. With a method of inoculating relatively mature plants

there is no need of resorting to a second generation of plants from seeds. Bluegrass is easily propagated vegetatively and by inoculation with a hypodermic needle a single clone may be inoculated over and over again until its resistance or susceptibility is definitely determined.

As a trial demonstration of the practical use of this method of progressive elimination of susceptible plants 50 seedlings about 2 months old were inoculated with the hypodermic needle using a suspension of fresh spores from grass. Some of the plants were rather small and were injured during the inoculation and only 36 survived. Of these, 10 plants became infected as follows:

34 days after inoculation,	5 plants infected = 13.9 per cent
37 do	8 do = 22.2 per cent
39 do	9 do = 25.0 per cent
77 do	10 do = 27.7 per cent

By this time the plants had formed new tillers so 78 days after the original inoculation the 26 noninfected plants were divided into 3 to 5 separate plants and each plant was reinoculated with the hypodermic needle as before. Whenever one or more plants from a clone were found infected the entire clone was considered susceptible and discarded. Considering each clone as a plant the results of the second inoculation are given on a cumulative basis as follows:

21 days after second inoculation,	12 plants infected = 33.3 per cent
24 do	14 do = 39.0 per cent
26 do	15 do = 41.1 per cent
29 do	16 do = 44.4 per cent
37 do	20 do = 55.5 per cent
58 do	20 do = 55.5 per cent

The remaining 16 plants were again divided into clones of 6 to 10 individual plants and reinoculated with spores from grass that had been incubated for three weeks at 35° C. in a moist chamber. Tests showed about 5 per cent germination in water. Within 44 days after the third inoculation 12 more clones had been eliminated, leaving only 4 clones not infected. Three weeks later the remaining four clones were inoculated with fresh spores from grass. Three of these were eliminated within 43 days, leaving only one clone. This was divided into 18 plants and inoculated.

No infection developed in 70 days, so 18 plants were reinoculated and one plant developed smut after 59 days, making 100 per cent infection, thus eliminating them all as susceptible after a total interval of one year and 6 days.

This method of inoculation may seem too slow to be practical, but it is believed that, with further study, it can be speeded up considerably. Most of these inoculations were made with chlamydospores having low germination percentages (none more than 5 per cent). Some success in increasing the percentage of germination of spores used for inoculation has been obtained by using Kreitlow's (7) heat treatment, but results have been erratic. With further trials using spores with better germinating records the percentages of infection from a single set of inoculations should be increased.

In many of the experiments reported the grass grew very slowly and the slow growth may have contributed to the long incubation period. Although the incubation periods have been discouragingly long, they are, as a rule, shorter following hypodermic-needle inoculation than after any other method. The shortest incubation period recorded is 13 days following hypodermic-needle inoculation, while the shortest for seed or soil inoculation is 36 days.

When routine inoculation tests are made and when the only consideration is the elimination of susceptible clones, it should be possible to combine hypodermic-needle inoculation with soil inoculation and shorten the time interval considerably.

The foregoing experiments do not constitute all of the inoculation tests that have been made, but they are considered representative of the results obtained to date. Some inoculation tests have, for no explainable reason, failed to produce any infection, but, with further practice and experience, results become consistently more successful. Thus, in an experiment started on May 17, 1944, in which 148 plants were inoculated with the hypodermic needle using fresh spores from grass, 48.6 per cent of the plants became infected by January 12, 1945, as a result of the original inoculation. This is considered to be a fair average of success with the hypodermic-needle method alone.

Further experiments are under way with the aim of perfecting the method for practical use as well as to learn more about the methods of infection and life history of the fungus in nature.

DISCUSSION

Ustilago striaeformis on bluegrass differs in many respects from that on other grasses as described by Davis, Fischer, and others. The formation of typical chlamydospores on artificial media has not been reported previously for this organism, and is not common in other smut fungi, although Wang (11) has described a similar phenomenon for *Ustilago crameri* Keke. Because of this ability of the fungus to complete its entire life history on artificial media it lends itself well to cytological and genetic studies. Preliminary studies of this nature are under way and will be reported later.

The failure of the fungus to produce the usual type of sporidia and the occurrence of infection from soil-borne inoculum long after the seedling stage are in contrast to previously accepted concepts of the life history of the species. Its behavior in this respect is probably correlated with the growth habits of bluegrass. In nature the chlamydospores most likely germinate in the soil, forming a mycelium that is capable of infecting the young tillers, as well as young seedlings.

SUMMARY

1. *Ustilago striaeformis* from bluegrass is readily cultivated on artificial media. Chlamydospores are formed on agar in great abundance and, al-

though some of them are slightly abnormal in size and shape, they germinate normally, producing cultures indistinguishable from those arising from spores produced on bluegrass.

2. Chlamydospores germinate by forming one or more branched germ-tubes that grow into a mycelium on nutrient agar. Two types of colonies occur; one that breaks up into sporidia-like fragments, and one that is typically mycelial. Both types of growth may produce chlamydospores, but certain cultures of each growth type have not sporulated.

3. Conventional methods of seed inoculation resulted in low percentages of infection. Relatively good infection was obtained by soil inoculation and by injecting chlamydospores with a hypodermic needle into the stem near the growing point.

4. Infection from the soil is not confined to the young coleoptile, but may occur on older plants, probably through young tillers.

5. The practicability of inoculating vegetatively propagated clones of bluegrass has been demonstrated. It appears to be a promising means of eliminating susceptible clones and identifying resistant ones in a program of selecting or breeding smut-resistant strains of bluegrass.

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TATTER LEAF OF SWEET CHERRY¹

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In mid-June, 1940, advice was requested in connection with abnormalities in sweet cherry fruits, purported to be of the Black Tartarian variety, in an orchard near Niagara Falls, Ontario. On inspection, the fruit was found to be slightly smaller than normal and appeared to be ripening prematurely. The fruit surface was somewhat roughened with shallow pitting, in many cases centered around tiny black specks. The foliage showed considerable interveinal pallor. This condition, which has frequently been observed on cherry leaves in the spring and which is usually outgrown as the leaves mature, is regarded as being induced by growth factors at present obscure. In addition to this "growth mottle," however, the trees in question showed a faint blotching and chlorotic flecking suggestive of a mild virosis. Examination of fruits and leaves failed to disclose bacterial or fungal parasites that might account for the disease. The following season, symptoms on trees in this orchard ranged from traces of mottling alone to mottling accompanied by more or less laceration.

PRELIMINARY TESTS OF TRANSMISSIBILITY

In June, 1940, small pieces of bark from the smaller branches of one of the affected trees were patch-grafted on the trunk of a two-year Elberta peach tree and on two suckers of a thirteen-year-old Black Tartarian tree in the laboratory orchard. The inoculated cherry tree, in 1941, put out dwarfed shoots and small, ruffled leaves up and down the main branch bearing the inoculated shoots and on the adjacent side of the next main branch. The remainder of the tree was normal in appearance throughout the 1941 growing season. Affected leaves were marked interveinally with brown flecks, rings, lines, and streaks which soon dropped out, leaving a lacy network of tissue very often involving almost the whole leaf. Most of the lacerated leaves were retained throughout the summer, though the effects of the disease gradually became obscured by the development of new leaves, almost normal in size and either without markings or with faint patterns of the banded or outline "oak-leaf" type. A similar sequence of events has taken place in this tree in the growing seasons of 1942, 1943, and 1944. By 1942, the disease had spread through most of the inoculated tree, and in 1943 and 1944, there were indications that its effects were gradually decreasing in severity. The fruit symptoms noted on the original tree have not been observed on the inoculated one and may not have been caused by this disease.

Further evidence that the disease is graft-transmissible, and thus of virus origin, was obtained from the young inoculated Elberta tree, many leaves

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of which were marked with small rings and chlorotic and necrotic spots early in May, 1941. These symptoms, which were the only ones apparent in the first season after inoculation, did not occur in 1942 and subsequent years, when a variety of other symptoms appeared later in the season on a number of leaves scattered through the tree. These included faint but definite mosaic and oak-leaf patterns (Fig. 1, D), rings, and a curious premature aging of the upper leaf surface (Fig. 1, C) which appeared as if covered with a very thin, finely checked, dull, dirty yellow encrustation.

Since 1940, the disease, for which the name "tatter leaf" (11) seems to be appropriate, has been seen in several orchards in the Niagara Peninsula. Hildebrand and Palmiter (11) have observed cases of tatter leaf on sweet cherries in New York State. A similar condition, called "lace leaf" and considered to be distinct from cherry mottle leaf, has been reported from Washington State by Reeves (9) on the varieties Bing, Napoleon, and Deacon. Cochran (4, 5) also records lace leaf on sweet cherry following inoculation with a ring spot of peach.

THE DIFFERENTIAL HOST RANGE

After the preliminary demonstration that tatter leaf was graft-transmissible, the virus was transferred by budding, in 1941 and 1942, to the several varieties of the genus *Prunus* comprising the range of the differential hosts used in studying the virus diseases of stone fruits (12) namely: *Prunus domestica* var. Italian prune, German prune, Lombard, and Reine Claude; *P. salicina* var. Abundance; *P. persica* var. Rochester, Elberta, and seedlings; *P. avium* var. Napoleon (Royal Anne), Bing, Black Tartarian, and seedling; *P. cerasus* var. Montmorency; *P. cerasifera*, Myrobalan seedlings; and *P. mahaleb*, seedlings. Throughout the experiments, the Black Tartarian tree inoculated in 1940 was the source of the diseased buds used in making inoculations.

METHODS

The transfer of the virus to the respective differential hosts was effected in two ways: by the double-budding technique (12) and directly to nursery stock or to orchard trees. In double-budding, a bud from the diseased tree and one from the desired host variety were inserted on each of three stock plants, usually peach or Myrobalan seedlings, which were cut back to the upper grafted bud the following spring. This method has the advantage over direct inoculation of nursery stock of permitting the use of differential varieties of clonal origin, each indexed on peach, concurrently with inoculation, to ascertain its freedom from virus infection. The varietal clones, on which symptoms are described below and which were used in double-budding, were healthy unless otherwise specified. The nursery stock may or may not have been healthy, as it was not indexed because of lack of indexing stock, but it appeared to be normal throughout the summer of 1942 before inoculation.

VARIETAL REACTIONS

On Italian prune.—In the double-budding experiment on peach stock in 1941, transmission was indicated by symptoms on the peach foliage. The prune, however, showed only a faint mottle on a few leaves in the three subsequent seasons.

On the nursery stock, an indistinct mottle was observed on leaves near the point of inoculation in the first year, but not in the second.

On German prune.—Only the double-budding technique was used with German prune. The peach stock indicated a positive transfer of the tatter-leaf virus, but the prune remained symptomless for three seasons after inoculation.

On Lombard plum.—On nursery stock, the diseased cherry buds established union with the Lombard, but the only symptoms observed on the plum were some faint irregular pale markings (Fig. 2, G) or slight necrotic spotting and shotholing on a few leaves in the first season. The next year, very few faint patterns appeared.

On Reine Claude plum.—Definite symptoms occurred on the peach stock double-budded with Reine Claude plum and tatter-leaf cherry in 1941, but the only indication of infection on the plum was a very faint mottle, sometimes approaching the oak-leaf type of pattern, and appearing annually in early summer.

On Abundance plum.—After double-budding on Myrobalan stock in 1942, Abundance shoots grew vigorously, but, like the varieties of *P. domestica*, were not much affected by the virus. In the two years since inoculation, some mid-season leaves were perforated with a few scattered pinholes, a feature not observed on Abundance in other series. According to the results of indexing on peach, the Abundance clone, though apparently symptomless, was carrying a virus of the peach-yellows-little-peach group.

On Rochester peach.—Inoculation of this variety was made in 1942 on nursery stock only. At the beginning of the 1943 growing season, slight superficial bark necrosis was observed near the inserted buds, and some leaves were definitely marked with small confluent rings, either dark green with pale centers (Fig. 1, A) or pale green with dark centers (Fig. 1, B), and mostly in the proximal three-quarters of the leaf lamina. On some leaves, the rings were larger and more irregular, and on others, part of the surface was chlorotic and dotted with small circular to oblong green islands. These patterns disappeared later in the summer when, except for some laceration of older leaves, other symptoms were wanting. In 1944, none of the early season symptoms appeared, but the foliage was somewhat dull and a few leaves were marked with the mid-season symptoms described on Elberta. The first crop of fruit, in 1944, was only slightly reduced, if at all.

On Elberta peach and seedling.—The reaction of Elberta nursery stock to tatter leaf was similar to that of Rochester but somewhat less pronounced, except for the laceration of older leaves, which was preceded by the formation of red-rimmed, fawn-colored, necrotic areas.

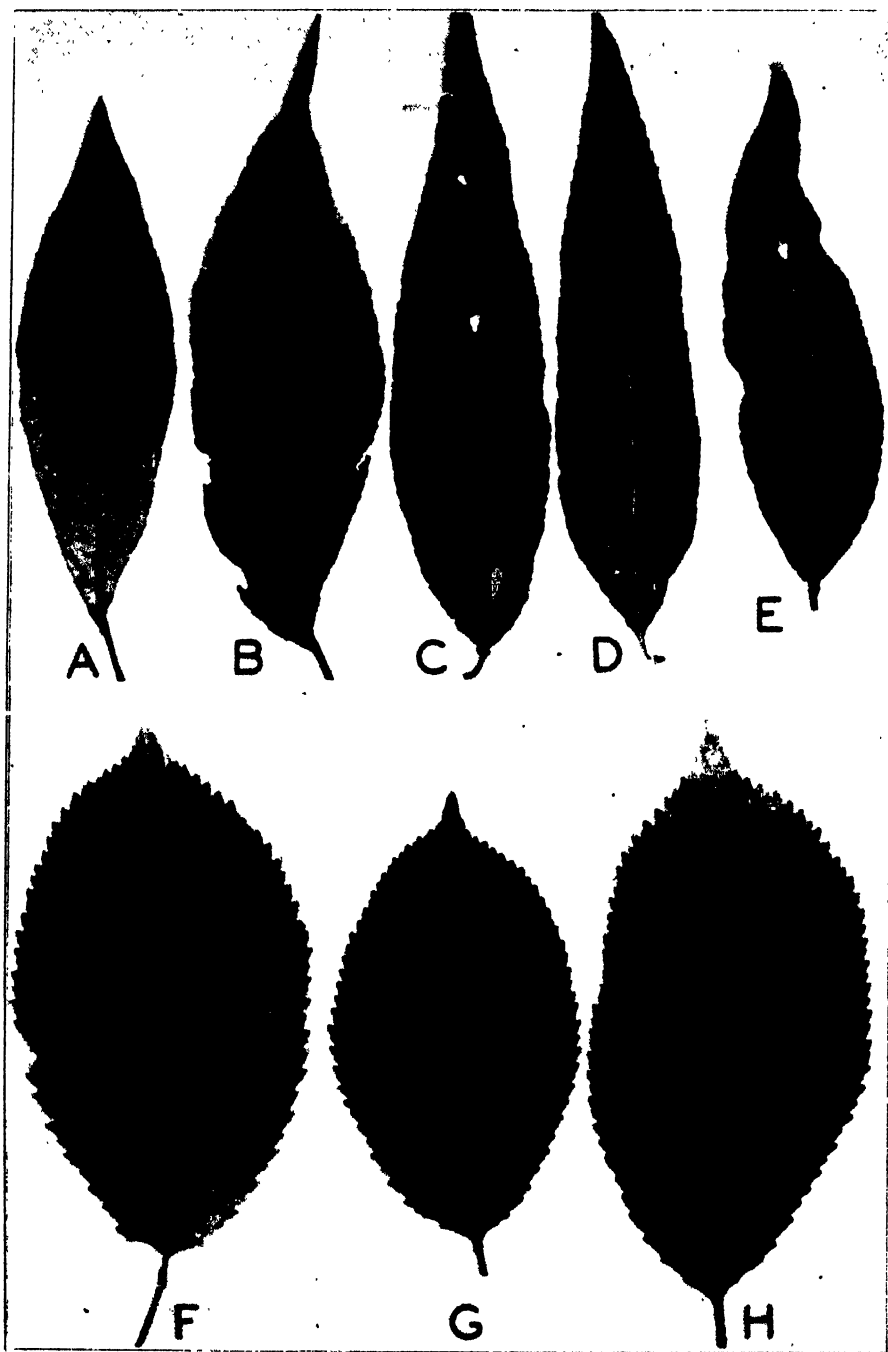


FIG. 1. Symptoms of tatter leaf on peach and Napoleon cherry. A-B. Acute symptoms on Rochester peach. C-E. Chronic symptoms on Elberta peach. F-H. Patterns and necrosis on Napoleon.

In the double-budding experiment of 1941, on the other hand, the characteristic early season symptoms were absent from the shoots both from the inserted Elberta bud and from the cut-back seedlings, but, as the leaves matured in the first season, their upper surfaces became somewhat lusterless and in some instances looked as though they had been lightly scoured with fine sand-paper near the margins. By the end of July, there had appeared the typical faint mosaic (Fig. 1, D), the surface aging (Fig. 1, C), and other patterns, which did not occur on the larger trees until the second summer. In addition, a number of leaves on most trees in this series were lightly sprinkled with a fine, persistent, red pin-spotting (Fig. 1, E). On the other hand, the fawn necroses and laceration of leaf tissue were wanting. The typical mid-season symptoms were also observed on these plants in the second and third years after inoculation. Some variations in the degree of symptom expression were noted on different peach seedlings.

On Black Tartarian cherry.—On nursery stock, some of the early leaves on a few spurs close to the inserted diseased buds were marked in the first season with fine brown lines (Fig. 2, A), roughly parallel to the main veins and outlining areas which later became necrotic (Fig. 2, B) and dropped out to give the typical lacy effect (Fig. 2, C, D). On Black Tartarian, the lacerations were intervinal and rarely if ever did they cross the main veins. Other leaves on the same spurs were faintly mottled with yellowish green. By the beginning of the second season, symptoms of both sorts were apparent on many leaves over about half the tree. In both years, leaves formed after the middle of June were symptomless. On the whole, tattering was much less severe on the nursery stock than on the orchard tree inoculated in 1940.

Late in August, 1941, a Black Tartarian tree of the same age as that used in the preliminary tests was inoculated by budding on shoots near the base of a main branch. Typical brown line markings and tattering were observed in the spring of 1942 but only on the inoculated shoot. The disease did not spread completely through the tree until 1944, when tattered leaves were relatively few, compared with those marked with a modified form of oak-leaf pattern. These symptoms appeared on leaves scattered here and there all over the tree.

On Napoleon cherry.—The only symptoms on Napoleon nursery stock in 1943 were observed early in the season near the point of inoculation. A few leaves were marked with small, pale green to chlorotic rings (Fig. 1, F), and some with larger, pale green rings or green-rimmed irregular pale areas (Fig. 1, G). Most of the patterns were faint and transient. A few necrotic spots, intervenial necrotic streaks (Fig. 1, H), and lacerations occurred on some leaves, but the tattering effect was less than on Black Tartarian. Some coarse, chlorotic lines and rings appeared in the second season over a larger area of the tree, but the necrotic markings and tattering were again inconspicuous.

On Bing cherry.—A small branch of a large, bearing tree was inoculated in August, 1941. The following spring, the first leaves to open on the inocu-

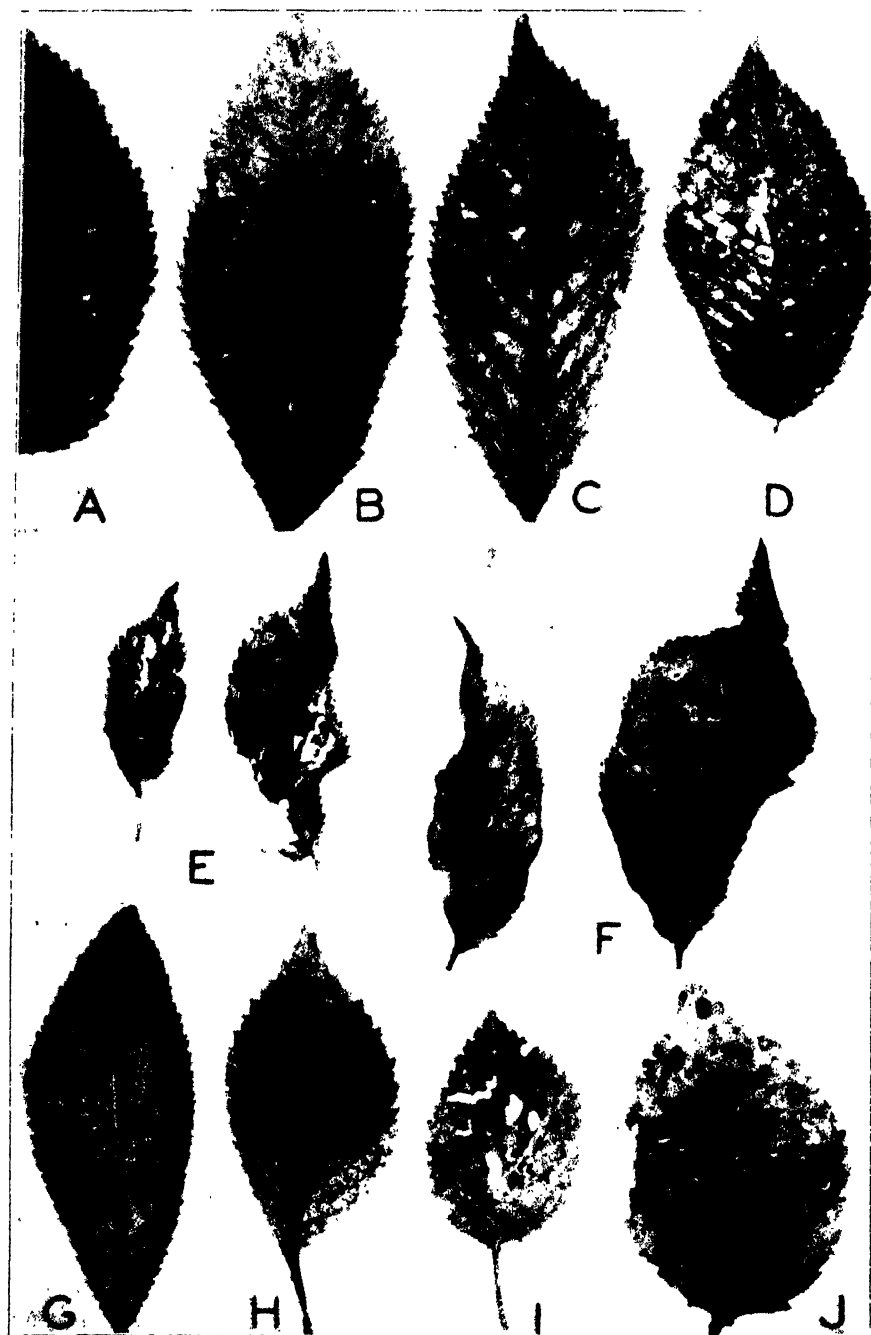


FIG. 2. Symptoms of tatter leaf on sweet cherry (Black Tartarian and Bing), on sour cherry (Montmorency), and on Plum (Lombard). A-D. Stages in the development of tattering on Black Tartarian. E-F. Necrosis, tattering, and ruffling in Bing. G. Mild mottle on Lombard. H-J. Ring and necrotic spotting on Montmorency.

lated shoot displayed some mottling and fine brownish etched lines quickly passing over into necroses (Fig. 2, F) and laceration (Fig. 2, E), which, instead of being interveinal as in Black Tartarian, involved larger areas of the leaf surface and often extended across main veins. Affected leaves tended to be ruffled and twisted (Fig. 2, E, F). No further symptoms appeared that season, but by the spring of 1943 the disease became evident on nearby branches, and by 1944 had spread throughout the tree. Symptoms in the second and third seasons varied from rings, mild patterns of the oak-leaf type, and mosaic mottling to necrosis, laceration, and distortion.

On a sweet cherry seedling.—A seedling of Elkhorn parentage was inoculated in the fall of 1941. Early in the next season, the seedling was cut back and the early leaves both of the seedling and of the inserted diseased Black Tartarian shoot were mildly but definitely mottled, but the characteristic lacerations failed to appear. In 1943 and 1944 a few leaves of both shoots were slightly tattered, but the predominating symptom was again the mild mottling, in either a mosaic or an oak-leaf pattern.

On Montmorency cherry.—The first symptoms to appear on the foliage of Montmorency nursery stock in the spring after inoculation were fine, translucent to dark brown, etched rings in all sizes up to about two millimeters in diameter (Fig. 2, I, I). The larger rings tended to be complete and regular, though frequently in tangential contact (Fig. 2, I). On the other hand, the smaller ones were often incomplete, confluent, and crowded to make a network tracery (Fig. 2, H). Numerous fawn-colored and usually isolated necrotic spots of various shapes and sizes, delimited by fine, red-brown lines (Fig. 2, J), were also present very early in the season, both on ring-spot leaves and on others. Most of the necrotic areas dropped out, but the ring spots, for the most part, either remained unchanged or gradually faded out. Symptoms were prevalent on foliage above the inoculation point, but were few below. The leaves produced from mid-June on were symptomless, so that, in late summer, the trees presented at first glance an almost healthy appearance, though, on closer examination, symptoms could still be seen on the older leaves.

In the second year, a different set of symptoms made their appearance at the beginning of the season, consisting of shiny lines and a trace of mottle on a few leaves, but there was little or no necrotic spotting. Much of the foliage put out up to the end of June was characterized by undulant margins, some rugosity, and more or less twisting of the laminae. As the season progressed, the new leaves developed more normally, and tended to obscure the general symptom picture.

On Myrobalan seedlings.—Although the tissues of buds and stock had united in several instances, so that transmission was possible, no symptoms were observed on Myrobalan seedlings in two years after inoculation.

On Mahaleb seedlings.—The leaves of the two Mahaleb seedlings on which union with the diseased bud had taken place exhibited a very faint mottle early in the first season and a variety of patterns, such as oak-leaf, coarse

lines and rings, and fine confluent rings, later in the summer. The latter symptoms reappeared on only one of the seedlings in the second year. A third Mahaleb seedling, on which the bud died shortly after insertion, remained symptomless both years.

DISCUSSION

Experience in recent years has shown that some plum and sweet cherry varieties and especially the sour cherry variety, Montmorency, can and frequently do carry virus without showing recognizable symptoms. Peaches, on the other hand, have so far been found to be free from masked viroses in nature, though it has been shown experimentally that they can act as carriers of certain viruses after having "recovered" from the initial phase of the disease. This characteristic of certain varieties of carrying a virus symptomless has to be taken into account in all experiments with virus diseases of stone fruits, however, to date, there has been no positive evidence that a masked virosis in a carrier has modified to any great extent the symptoms induced by another virus transmitted to that host. Consequently, symptoms manifested by a variety after, but not before, inoculation can be considered to be the result of that inoculation.

The plum varieties tested so far, as well as the Myrobalan stock, have been little affected by tatter leaf. Any indications of transmission have been so tantalizingly indefinite that transfers from inoculated plum varieties back to cherry and peach will have to be made for confirmation.

There were some apparent discrepancies in the symptom picture on peaches with regard both to the early and to the mid-season symptoms. It should be noted that the rings and chlorotic markings, which, occurring only at the beginning of the first season after inoculation, may be regarded as the acute phase of the disease, *cf.* McKinney and Clayton (8), were found on the nursery stock but not on the double-budded seedlings which were cut back. It has also been observed, in connection with a number of other stone-fruit viruses under investigation, that the acute symptoms which they normally induce on unpruned peach seedlings have been suppressed by cutting the seedling back to the bud. There is no reason to believe that the movement of the tatter-leaf virus in the host differs essentially from that of other viruses which is considered to be in the direction of the flow of the main stream of elaborated foods (1, 2). Accordingly, after inoculation in the fall, the virus would tend to move out into the tissues in the immediate vicinity of the inserted bud and possibly below it, and to remain there until carried to the upper part of the tree when movement of food materials and growth are resumed in the spring. Indeed, the distribution of symptoms in Montmorency suggests that little movement of the virus takes place until spring. McKinney and Clayton (8) consider that the developmental stage at which tissue becomes infected tends to determine the type of disease reaction. If, as suggested by Valteau (10) in connection with ring spot of tobacco, the acute phase is due to the infection of already differentiated tis-

sues and the chronic phase follows invasion of undifferentiated embryonic tissue, it would appear that the tatter-leaf virus did not move out into the upper buds of either seedling or larger tree until after the first leaves were partly formed. In support of this view was the tendency for the acute symptoms to be located in the basal three-quarters of affected leaves rather than in the tips. Cutting back the inoculated seedlings, besides circumventing this development, either forces into growth already infected buds close to the graft or induces the production of adventitious shoots from infected tissue. The early appearance of chronic symptoms in the first season on cut-back seedlings can be accounted for by the development of shoots from infected meristems, supplemented by the comparatively rapid multiplication of the virus during the very vigorous growth induced by the cutting-back operation. On the other hand, in the larger trees where chronic symptoms were delayed until the second season or later, the slower accumulation of the virus to the degree of concentration and distribution necessary for symptom expression, was probably due to the relatively smaller dosage of inoculum, only one or two diseased buds being used in inoculation, and in some measure to the less rapid multiplication of the virus incident to less vigorous growth.

Some differences in the reaction of sweet cherry varieties to tatter leaf were indicated. Napoleon appeared to be the least susceptible, exhibiting chlorotic ring, line, and other patterns, and comparatively little necrosis and tattering. Bing, on the other hand, was more affected by tattering than was Black Tartarian, particularly with respect to the size of the necrotic areas on individual leaves. While Black Tartarian appears to occupy an intermediate position of susceptibility, its behavior after inoculation has been somewhat anomalous, both in the severity and extent of tattering and in the rate at which infection spread through individual trees. It may well be that the differences observed should be attributed to the time of year at which infection took place rather than to the vagaries of the variety itself. The orchard tree, in which violent symptoms of tattering appeared over about a third of the tree in the first season and which was almost completely infected the next year, was inoculated in June when active growth was in progress. All other Black Tartarian trees and those of other cherry varieties were inoculated in late July or early August after growth had slowed down. Symptom expression in these trees was restricted the following spring to shoots near the point of inoculation and at least two more growing seasons were required for the disease to permeate each tree. It is therefore reasonable to suggest that both the movement and the multiplication of the virus in the host were more extensive after the early summer infection than after the later ones.

The rôle of foliar necroses and lacerations in the symptom expression of tatter leaf on sweet cherries is not entirely clear at present. On the grounds that the tree from which the virus was originally obtained had no such symptoms and may have had the disease for a number of years, it may be argued that the tatter-leaf condition is part of the acute phase. A similar conclusion

is suggested by the almost complete absence of tattering both on the sweet cherry seedling inoculated in 1941 and on the Black Tartarian budded thereon, if it be assumed that cutting back suppresses acute symptoms in sweet cherries as it does in peaches. Furthermore, the evidence so far obtainable from the inoculations of nursery stock and of larger trees, while inconclusive, is not incompatible with the theory. On the other hand, recurrence of tattering on the Black Tartarian inoculated in June, 1940, indicates that the so-called acute symptom, though gradually waning, may have almost the persistence of a chronic one. It seems probable that the time of year at which infection occurred and possibly a higher level of virus content may have been factors in prolonging the production of an acute symptom. Whether or not the necroses and lacerations are shown to be the acute symptoms and the non-necrotic patterns, the chronic ones, both types are manifest on sweet cherry only on leaves produced in the early part of the summer. Additional data are necessary to determine how much this seasonal peculiarity is due to external factors, as for example temperatures during leaf development (7), and how much to internal factors, such as the rate of movement and multiplication of virus within the host.

The etched rings and necrotic spotting which constitute the acute symptoms of tatter leaf on Montmorency, bear considerable resemblance not only to the acute symptoms of the more virulent strains of prune dwarf (12), but also to those of necrotic ring spot of sour cherry (3, 6) on the same host. This similarity of symptoms can be explained by assuming either that each of the diseases in question is caused by one of three closely related single viruses, or that one or more is caused by a virus complex, one component of which is common to all three, or that Montmorency can react with much the same response to more than one distinct virus. Though the problem of possible relationships thus posed cannot be fully resolved from the data at present available, some deductions can be made. The distinct differences between the symptoms of tatter leaf and those of prune dwarf on other varieties of the range of differential hosts is an argument against the close relationship of the causal viruses and in favor of the theory of like host response to unlike causes, provided of course that a virus complex is not involved in one or both diseases. Up to the present, differential symptom expression on the range of hosts used in the experiment and particularly on the peach, leads to a similar conclusion, regarding tatter leaf and necrotic ring spot, which is supported by the following piece of circumstantial evidence. Subinoculation to clonal yearling Italian prune trees from the Montmorency, Napoleon, and Black Tartarian nursery stock, to which tatter leaf had been previously transmitted, demonstrated that the Montmorency carried a second virus which was not present in the other two varieties and which in all probability was one of the forms of necrotic ring spot commonly occurring in masked form in Montmorency. Since the inoculation with tatter leaf induced striking symptoms on an already diseased Montmorency, the introduced virus was evidently not the same as the inherent one. On the other hand, one of

two Black Tartarian trees inoculated with necrotic ring spot from sour cherry had, in two seasons, leaves with symptoms resembling those of tatter leaf, though, on the whole, the sweet cherry was much less affected by the necrotic-ring-spot virus than by the tatter-leaf virus. Hildebrand also reported (6, 11) that the sour cherry ring spot induced tatter-leaf symptoms on sweet cherry. It is possible that the necrotic-ring-spot virus he used was more closely related to the tatter-leaf virus than the Canadian strain seems to have been.

In so far as prune dwarf, tatter leaf, and necrotic ring spot of sour cherry can be separated as three distinct viroses, on the basis of their symptom expression on a common range of differential hosts, it is necessary to postulate the existence of at least three distinct viruses or virus strains, whether they act singly or as part of a complex. If they are related and how closely they are related are vexed questions which must await the outcome of further research.

SUMMARY

Tatter leaf of sweet cherry is a virus disease named for its most striking symptom on that host, and has been found in several orchards of the Niagara Peninsula in Ontario. One strain of the causal virus has been transmitted to a range of hosts comprising several varieties of plums, peaches, and sweet and sour cherries.

The disease has little apparent effect on plum varieties, some of which react with an indistinct mild mottle on early leaves.

On peach, the acute symptoms (slight superficial bark necrosis, ring patterns, and chlorotic markings) appear at the beginning of the first growing season after inoculation. Subsequently, usually in the second year, chronic symptoms including faint mosaic and oak-leaf patterns, dullness and premature aging of the upper leaf surface, fine red pin-spotting, and occasionally red rim, fawn necroses, are to be found on leaves scattered over the tree. Acute symptoms are suppressed in favor of the chronic symptoms when seedlings inoculated in late summer are cut back to the bud the following spring.

The first symptoms to appear on Black Tartarian cherry trees the spring after inoculation are fine brown lines circumscribing interveinal areas which soon become necrotic and drop out. Faint yellowish mottling and oak-leaf patterns also occur. Symptom expression appears to be confined to leaves emerging early in the growing season and to vary in intensity and extent with the time of year at which infection takes place, becoming less intense and more restricted in distribution the later the infection. Necrosis and laceration affect Napoleon to a lesser and Bing to a greater degree than Black Tartarian.

Fine etched rings and necrotic spotting form the acute phase, and undulations, rugosity, and twisting of the leaf laminae the chronic phase on the sour cherry variety, Montmorency. The acute symptoms resemble those of ne-

erotic ring spot and some strains of prune dwarf on that variety, but the relationships, if any, of the causal viruses are as yet a matter of conjecture.

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ANNOUNCEMENT

Hotel headquarters for the meetings of the American Phytopathological Society at St. Louis, Missouri, March 27-30 will be announced in the February issue of PHYTOPATHOLOGY. Assignments by the American Association for the Advancement of Science were not announced before the January issue went to press.

Abstracts of papers to be presented at the meetings of the Society are due in the Office of the Secretary not later than January 25, as was announced in the December issue of PHYTOPATHOLOGY.

EDWARD FRANKLIN GAINES

1886-1944

HARRY B. HUMPHREY AND FREDERICK J. STEVENSON

Edward Franklin Gaines was born January 12, 1886, near Avalon, Missouri. When a lad of fourteen the family moved from Missouri to a farm near Chewelah, in the Colville Valley of northeastern Washington. It was here and in Missouri that he received his public school education. Thereafter he attended the State Normal School at Cheney, Washington, where he was graduated in 1907. We next find him as the principal of a grade school in Ritzville. In 1909 he entered the State College of Washington, at Pullman, where, in 1911, he received the degree of Bachelor of Science in Agriculture.

On graduation the subject of our narrative was appointed to a position on the Experiment Station staff, as assistant cerealist. The nature of this appointment enabled him to continue his studies to the end that in June, 1913, he was awarded the M.S. degree. We next find him for a semester in 1915 at Harvard University as a graduate student of genetics and plant breeding under the leadership of the late Dr. E. M. East. Returning to Pullman, he resumed his duties as assistant cerealist, which position he filled until 1917, when he was promoted to the rank of cerealist. In 1920 he was again granted leave for resumption of his postgraduate studies at Harvard, where, in 1921, he received the degree of Doctor of Science.

By way of partial acknowledgment of the outstanding quality and character of Dr. Gaines as student and man, it suffices here to mention the fact that, while at Pullman, he was elected to membership in Alpha Gamma Rho, Alpha Zeta, Phi Kappa Phi, Sigma Xi, Phi Sigma, and Phi Beta Kappa. Of these honors and badges of student esteem and distinction he was justly proud, yet he was ever the same wholesomely modest and genuine student, and inspiring teacher.

His ability as a teacher was early recognized, and he served his alma mater eminently, first as instructor in agronomy (1911-1917), then as assistant professor of farm crops (1917-1921), and associate professor of farm crops (1921-1930), and finally as professor of genetics in agronomy, from 1930 until his death. Because of illness, he was unable to be at his desk during the last nineteen months of his life.

The last six months of 1930 Dr. Gaines spent in Europe, where he visited experiment stations and educational institutions in Great Britain, Russia, Germany, Sweden, and Denmark. Incidentally, while in Europe, he attended the Fifth International Botanical Congress, at Cambridge, and the Second International Congress of Soil Science, held that summer in Leningrad and Moscow. In early October he took up residence in Halle, Germany, seat of the Institute for Plant Breeding and Plant Research.



EDWARD FRANKLIN GAINES
1886-1944

Here he visited and made observations on the several field experiments and had access to the records. He also made several excursions into the surrounding country, where he visited private plant-breeding centers, outlying stations of the Institute, centers of beet-sugar production, and several large, privately owned farms.

Most important among Dr. Gaines' contributions as a geneticist and plant breeder are his papers on the genetics of bunt resistance in wheat and his studies on the inheritance of smut resistance in wheat and oats. The appended list of his published research will afford the reader a ready grasp of the range and importance of his scientific contributions to our knowledge of agronomy, and of genetics as applied to breeding for disease resistance.

During his long term of service as a teacher students came to Dr. Gaines from Canada, Belgium, Germany, the Philippines, China, and Japan to study under his supervision. He was visited by geneticists from every continent, men who desired particularly to learn of his methods and results.

To his intimate friends "Ed" Gaines was more than a great scientist. He was a great man. He believed in mankind and in his social betterment, and was always to be found in the front ranks of character-building organizations. He worked with young people in the Boy Scouts and in the Church, with students in various college organizations, especially in the Alpha Gamma Rho fraternity, and with farmers, in whose welfare he always showed, through the Grange, an intense interest.

He radiated trustworthiness and steadfastness of purpose. He had the ability to undertake worthwhile projects and carry them through, often in spite of formidable opposition.

Those students privileged to work under his direction were treated not as inferiors but as close friends whom he inspired with his own zeal for science and right living. He was not an outstanding lecturer, but he was an eminent teacher and leader. Not many words but more doing seemed to be his motto.

His scientific achievements will long be remembered, but his influence for good over many lives, while not so easily evaluated, probably will result in greater gains to society than the results of his scientific work. We can truly say that the Pacific Northwest and the whole world are enriched physically and morally because Ed Gaines passed this way.

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CHEMICAL CONTROL OF SEED-BORNE FUNGI DURING GERMINATION TESTING OF PEAS AND SWEET CORN¹

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In routine germination testing in the laboratory seeds usually are placed close together on moistened paper towels. Since the atmosphere in the germinators is very humid, bacteria and fungi grow rapidly and spread quickly from infected to noninfected seeds and seedlings. Because the seeds are crowded, decays caused by seed-borne pathogens may be very severe in the germinator. *Rhizoctonia solani* Kühn originating in a few infected beans or peas (4) often grows throughout, and rots most of the seeds in, a rolled towel in 5 to 7 days. Molds frequently develop in lots of beans, corn, and peas (4, 5), and especially in seeds of low vitality, to such an extent that the seedlings become hidden and can be observed only with difficulty.

The fungicides commonly applied to seeds prior to planting (2, 5, 6, 7) have been used to control both pathogens and saprophytes troublesome in laboratory germination testing. Hay (5) reported that certain organic mercurials controlled the molds on bean seeds very satisfactorily and that the laboratory germination was uniformly higher for treated than for untreated seeds. Porter (9) found that the volatile, water-soluble mercurials were very effective as laboratory seed disinfectants and disinfestants. Crosier and Patrick (4) concluded that mercuric chloride, and dusts containing ethyl mercury phosphate, as well as several insoluble organic mercurials prevented mold growth if the germination periods did not exceed 10 days.

Certain copper compounds including copper oxalate, copper oxychloride, and cuprous oxide were not so effective as the mercurials in short-period tests. Buchholtz (2) stated that the chemicals (copper carbonate and ethyl mercury phosphate) recommended for control of sorghum smut also killed molds that developed on seeds during storage. Patrick and Crosier (8) found that any one of several mercurials, when used either as dip or dust, controlled *Alternaria* spp. and *Rhizopus nigricans* Ehr. in germinating cabbage and radish seed. Cuprous oxide and zinc oxide, however, caused only slight reductions in the percentages of fungus-seed associations. Hoppe (6) found that Arasan reduced the *Diplodia zeae* (Schw.) Léov. on infected corn seed but that Spergon was ineffective in control of this fungus. Muskett and Colhoun (7) reported that Arasan (or Nomersan) eliminated *Colletotrichum lini* (Westerd.) Toch. and partially controlled *Polyspora lini* Laff. in flax seed. They concluded that the organic mercurials were effective only when used as short wet treatments.

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METHODS AND MATERIALS

The present investigation was undertaken to determine the relative fungicidal values of Arasan, Ceresan, du Pont 1452 C, Semesan Jr., Spergon, and United States Rubber Compound No. 604. All chemicals were applied by agitating weighed amounts of dust with measured quantities of seeds in glass jars secured to a rotating wheel treater. The treatment periods were uniformly 5 minutes. A quantity of Stowell's Evergreen Sweet Corn grown in 1942 contained about 8 per cent of seeds infected with *Diplodia zeae*. *Rhizopus nigricans* and species of *Aspergillus*, *Fusarium*, and *Penicillium* occurred as contaminants on 50 to 60 per cent of the seeds. This lot of sweet corn seed was treated with certain fungicides at the following rates: Arasan (50 per cent tetramethyl thiuramdisulfide), 0.15 per cent by weight (1½ oz. per bu. of seed); Du P. 1452 C (7.7 per cent ethyl mercury p-toluene sulfonamide), 0.06 per cent by weight; Semesan Jr. (1 per cent ethyl mercury phosphate), 0.22 per cent by weight; U.S.R. No. 604 (essentially 2,3-dichloro-1,4-naphthoquinone), 0.11 per cent by weight. The seeds were germinated in rolled paper towels, placed in humid germinators with either constant temperatures of 25° C. or alternating temperatures of 20° and 30° C. The seedlings were removed and the fungicidal treatments were evaluated 6 days after initiation of the tests.

A sample from the 1942 crop of Thomas Laxton peas was separated into six portions and treated with chemical dusts applied in percentages by weight of the seeds as follows: Arasan, 0.15 per cent (1.44 oz. per bu.); Ceresan (2 per cent ethyl mercury chloride), 0.20 per cent; Semesan Jr., 0.20 per cent; Spergon, 0.20 per cent; and U.S.R. No. 604, 0.10 per cent. The seeds were germinated at 20° C. Records were taken the sixth day of germination.

The treated lots of peas and sweet corn were separated into 100-seed samples and distributed in triplicate to 11 official seed analysts in New York and other states for germination and disease studies. The peas were also planted at Geneva, N. Y., in greenhouse soil infested with *Pythium ultimum* Trow., *Rhizoctonia solani*, and *Fusarium* spp. The treated peas were planted in 5 replicates of 100 seeds, the untreated peas in 10 replicates of 100 seeds. After 25 days the emerged seedlings were counted, examined, and cut at the soil line, and the green tops were weighed.

RESULTS

Since the sweet corn seedstock was heavily infested with *Rhizopus nigricans* several cooperating analysts stated that the untreated germinated seeds were entirely obscured by growths of this fungus. Neither the statements nor the data of any analyst indicated that *R. nigricans* depressed the actual germination. This is in accord with previous findings at the New York laboratory. In experiments over a period of six years seedstocks infested with *R. nigricans* alone have not produced more seedlings from treated than from untreated seeds. Control of *R. nigricans*, however, may be accom-

panied by an increase in the green weights of the germinating seeds and seedlings. The results of the present investigation (Table 1) with a stock of sweet corn in which *Diplodia zeae* was also a factor suggest that increased weight of seeds and seedlings is obtained from even a partial control of molds.

Diplodia zeae grew rapidly on the untreated infected seeds and destroyed or injured from 3 to 10 per cent of the seedlings. Material was examined

TABLE 1.—*Influence of certain seed protectants upon seed germination, incidence of fungus contamination or infection, and the green weight of Stowell's Evergreen sweet corn seedlings*

Seed protectant	Germination		Seeds or seedlings with growths of								Green weight of seedlings	
	Normal	Total	<i>Rhizopus nigricans</i>		Pen., Asp., and Horm. ^a		<i>Fusarium</i> ^b		<i>Diplodia zeae</i>		No.	Gm.
	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.
Arasan												
Times first		2		2		3½		3½		7½		1
Times excelled ^c		29		22½		19½		28		40½		19
Average	68	78		28		2		8		2		66
Du Pont 1452 C												
Times first		1½		6		2		2½		3		8
Times excelled		29½		37		25½		30		29		39½
Average	71	78		24		5		6		4		70
Semesan Jr.												
Times first		3		2		2½		1		½		1
Times excelled		27½		20½		24		19		20½		25
Average	70	78		32		6		8		5		66
U.S.R. No. 604												
Times first		1½		1		3		3½		0		0
Times excelled		23		27		31		29		20		15½
Average	69	77		28		2		5		6		65
No treatment												
Times first		0		0		0		1		0		1
Times excelled		1		3		0		6		0		11
Average	60	74		56		14		17		15		60

^a Species of *Penicillium*, *Aspergillus*, and *Hormodendrum*.

^b *Fusarium moniliforme* was the most frequently observed species.

^c Number of times this material excelled all other materials and the check, 11 possibilities. Ties of 1 material with 1 to 4 others are recorded as ½ to ½, respectively.

^d Number of times this material excelled one of the other materials or check, 44 possibilities for each material.

before the hyphae spread extensively throughout the rolled towels, hence the injury from *D. zeae* was limited to the infected seeds and those immediately adjacent. The increased percentages of germination (Table 1) in the treated 100-seed lots are undoubtedly attributable to the chemical control of *D. zeae*.

Only U.S.R. No. 604 effected more than a trace of chemical injury. The average percentages of germination from the seeds treated otherwise (Table 1) indicate a general absence of phytotoxicity. The organic mercurial, Du P. 1452 C, was outstanding in the control of molds and *Diplodia zeae*, although U.S.R. No. 604 and Arasan were nearly as fungicidal.

The seeds treated with Du P. 1452 C produced a greater weight of sprouts than seed treated with any other material in trials by eight cooperators. The weight of the sprouts detached from the endosperms and seedcoats averaged 54.3 grams for the 100 seeds treated with Du P. 1452 C or a gain of 23 per cent over the 44.3 grams for the untreated seed. The plumules and radicles frequently were larger in the former tests. A heavier production of lateral

TABLE 2.—*Influence of certain seed protectants upon seed germination, fungus- and bacterial-contamination and green weight of Thomas Laxton pea seedlings*

Seed protectant	Germination		Seeds or seedlings with growth of						Green weight of seedlings	
	Normal	Total	<i>Rhizopus nigricans</i>	Pen., Asp., Fus., etc. ^a	Bacteria ^b				No.	Gm.
	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	
Arasan										
Times first		1½	5	2		0		5½		
Times excelled ^d		32	31½	33		22½		44½		
Average	87		93	2	1		6			116
Ceresan										
Times first		3	5	2½		2½		1½		
Times excelled		31	26½	34½		32½		34		
Average	89		95	4	1		5			114
Semesan Jr.										
Times first		1	2½	1		3		½		
Times excelled		19	31½	21		26½		17½		
Average	86		93	3	3		5			107
Sperguson										
Times first		3½	3½	1½		½		1½		
Times excelled		29½	41	36		27½		24½		
Average	88		94	2	1		5			111
U.S.R. No. 604										
Times first		2	1½	3		3½		1½		
Times excelled		34	33½	40½		38		22½		
Average	88		95	2	1		3			111
No treatment										
Times first		½	0	0		1½		½		
Times excelled		18½	0	0		18		22		
Average	85		95	9	8		6			108

^a Species of *Penicillium*, *Aspergillus*, *Fusarium*, and other fungi.

^b *Erwinia carotovora* caused most of the soft-rotting.

^c Number of times this material excelled all other materials and the check, 11 possibilities. Ties of 1 material with 1 to 5 others are recorded as ½ to 5, respectively.

^d Number of times this material excelled one of the other materials or check, 55 possibilities for each material.

roots also was observed. The average weight per detached seedling was also greatest for the lots treated with Du P. 1452 C. Since the stock of peas used in this study contained very few dead, weak, or contaminated seeds, the control of molds and bacteria was complete in several tests and nearly so in the others. Each cooperator reported that the incidence of *Rhizopus nigricans* and of *Penicillium* spp. was less in every treated lot than in any untreated lot of seeds. Sperguson and U.S.R. No. 604, excelled in the control of mold fungi (Table 2) while the latter also ranked first as a protectant against

bacterial contaminations. The chemical control of the bacteria and fungi greatly facilitated the examination of the germinant seeds.

It is obvious that growth of bacteria and fungi in the untreated seed was too limited to depress the germination greatly. For this reason the treated seeds produced only slightly more normal and total sprouts than did the untreated. Unfortunately the stock of peas was not infected with *Ascochyta pisi* Lib., *Rhizoctonia solani*, or *Sclerotinia sclerotiorum*, so the value of the chemicals in eliminating these pathogens was not determined.

In 55 comparisons with other protectants (Table 2) Arasan excelled, or tied, 46 times on the basis of total green weight from 100 seeds and seedlings. Each cooperator who measured the length of radicles and plumules reported that Arasan excelled all other treatments. By the same criterion Ceresan appeared to be only slightly inferior to Arasan both in the present study and in former experiments with similar seedstocks. Ceresan had previously

TABLE 3.--*Emergence and green weight of tops of plants from treated and from untreated pea seed planted in contaminated greenhouse soil*

Seed protectant	Plants emerged in 25 days			Green weight of tops	
	Normal	Weak or infected	Total	Total	Gains ^a
	Per cent	Per cent	Per cent	Grams	Per cent
Arasan	85	4	89 ± 5	116	332
Ceresan	39	11	50 ± 8	66	144
Semesan Jr.	37	5	42 ± 3	50	85
Spergon	86	3	89 ± 9	120	348
U.S.R. No. 604	76	6	81 ± 3	103	283
None	26	8	34 ± 7	27	

^a The weight of plants from untreated seeds equals 100 per cent.

(4) excelled the copper compounds in this respect with germination tests of peas terminating within seven days.

In greenhouse beds both Arasan and Spergon were slightly superior to U.S.R. No. 604 (Table 3) in the protection of pea seeds and seedlings against damping-off. The water-soluble ethyl mercurials, Ceresan and Semesan Jr., did not compare favorably with the former materials as seed protectants. It should be noted that Arasan was applied at only three-fourths and U.S.R. No. 604 at one-half the weight dosage of the other three materials. The plants in each of the replicates from treated seed were larger and more uniform than in those from untreated seeds. The average weight per plant (Table 3) indicates that each of the materials protected the emerged seedlings from root-rot and other fungus injury. The plants from the Arasan- and Spergon-treated seeds were taller and were maturing more rapidly than those grown from the peas left untreated or treated with other materials.

DISCUSSION

The organic mercurials, Ceresan and Semesan Jr., applied to seed corn prior to planting have effected practical control of molds in laboratory

germination techniques. Low-germinating stocks of seed, such as the sweet corn seed used in this study, may be so infested with sub-surface growths of *Rhizopus nigricans*, however, that seed treatment does not eliminate all of this fungus. The dosages of the seed protectants may be considered minima for application to normal planting stocks of sweet corn. Slightly greater amounts of Semesan Jr. will adhere to the seed coats and are usually recommended, while the dosages of the other materials could be increased from 2 to 5 times. However, with the exception of Arasan and Spergon, and possibly of Du P. 1452 C, such increases in dosage would be accompanied by definite phytotoxicity. It would appear then that any of the materials used in this study could be applied in sufficient amounts to control *R. nigricans* and other fungi but that Arasan alone would effect a high degree of control of both pathogenic and saprophytic fungi without injury to the seedlings. Arasan has frequently been applied without dilution to corn seed and the seedlings developed normally. Usually the adherence rates varied from 0.30 to 0.45 per cent by weight with an average of 0.35 per cent (3.1 ounces per bushel of seed).

Under certain conditions the maximum adherent dosage of Arasan may be necessary to inhibit *Diplodia zeae*. Hoppe (6) employing a technique which permitted maximum adherence of dusts to corn seed found Arasan about as efficient as New Improved Semesan Jr. or Barbak D in the inhibition of *D. zeae*. Since the dosage of Arasan used by Hoppe was probably 50 to 100 per cent heavier than those of the other dusts it may be inferred that equal dosages would prove Arasan to be relatively less efficient. Repeated experiments at the New York State laboratory, however, indicate that the application of a mixture of equal parts of Arasan and tale (only 25 per cent tetramethyl thiuramdisulfide) to infected corn seed inhibits the growth of *D. zeae*.

The material Du P. 1452 C is, in certain respects, preferable to Arasan for laboratory practice, since it can be used as an instant dip. The consistently high green weight of seedlings (Table 1) from Du P. 1452 C-treated seed has been observed repeatedly in other experiments. It is not uncommon for the seedlings from each test of treated seed in a series of 20 samples to outweigh the germinated seed in the untreated control by 5 to 10 per cent. When applied to commercial seed usually less heavily contaminated than the stock of Stowell's Evergreen sweet corn used in this trial, a 0.20 per cent suspension of Du P. 1452 C in water or a 20 per cent Du P. 1452 C-80 per cent flour mixture practically eliminated the *Rhizopus* and *Penicillium* and inhibited the development of *Diplodia zeae* and *Gibberella zeae* (Schw.) Petch.

In the control of molds U.S.R. No. 604 is about equal to Arasan at the dosages used in this study. At equal dosages U.S.R. No. 604 might prove the better. However, while Arasan causes no seedling injury at heavy dosages, U.S.R. No. 604 appears to be phytotoxic if the dosage exceeds 0.11 per cent by weight. In the control of *Diplodia zeae* this material is definitely inferior

to Arasan and to the organic mercury dusts. In this regard U.S.R. No. 604 appears similar to the related quinone compound, Spergon, which did not inhibit the growth of *D. zeae* on infected seed (6).

New Improved Ceresan, one of the standard disinfestants for pea seeds, can be applied in either dip or dust form and is effective at dosages only 20 to 40 per cent as heavy as for most other materials. Semesan Jr. at five times the dosage rate of New Improved Ceresan has often been substituted for it since Semesan Jr. contains the same fungicidal ingredient and adheres at the correct rate to control seed contaminants without producing seedling injury.

Ceresan and New Improved Ceresan have been of some value in controlling *Ascochyta pisi* Lib. in seedstocks of peas and winter vetch (3, 4). Bayles *et al.* (1) also found that the organic mercurials reduced the amount of seed-borne *Ascochyta*, while cuprous oxide was ineffective.

Since the advent of Spergon as a seed protectant for peas, a mixture of 60 per cent Spergon and 40 per cent flour by weight has been used in the laboratory. Such a dust adheres at a rate equivalent to 2 to 4 ounces of Spergon per bushel of seed. The results of this study indicate that Spergon applied at about this dosage will be equal or even superior to Semesan Jr. in the control of molds. Spergon seemed to cause no discomfort to the worker when treating seeds, while the other four dusts applied to pea seeds in this investigation caused slight irritation. Seedstocks of pea treated with Spergon before their receipt at the seed testing laboratory usually produced strong clean seedlings on paper towels. Heavily dusted seeds have germinated normally although the coverages indicated that Spergon had been applied at rates of 5 to 8 ounces per bushel.

Arasan has eliminated molds from germinant pea seeds treated at the canning factory, and Arasan has been practically equal to Spergon in this respect. Similar studies with cabbage seed (8) have indicated that both Arasan and Spergon are about equal to Semesan in control of *Alternaria* spp. and *Rhizopus nigricans*.

SUMMARY

Every chemical preparation used in this study, and especially Du P. 1452 C, inhibited, or partially controlled, the seed-borne fungi of sweet corn in germination testing. Increases in germination attributable to seed treatment paralleled the control of *Diplodia zeae* but probably not the control of *Rhizopus nigricans*.

Only U.S.R. No. 604 caused any chemical injury detectable by size or development of the sweet corn seedlings. Every other material resulted in increased green weight of the combined seeds and seedlings. Du P. 1452 C excelled or equalled the other chemicals in 41 of 44 possibilities on the basis of green weight. Arasan was the most, and U.S.R. No. 604 the least, efficient material in preventing *Diplodia zeae* from developing on seeds and from destroying seedlings.

The chemical protectants were effective in controlling molds and bacteria on pea seeds and seedlings, although U.S.R. No. 604 slightly excelled the other materials. Only Semesan Jr. failed to effect a slight increase in both the germination of seeds and green weight of seedlings. On the basis of green weight of seeds and seedlings Arasan and Ceresan excelled U.S.R. No. 604, Spergon, and Semesan Jr.

Arasan and Spergon permitted only 5 per cent of the pea seeds to decay in soil naturally infested with *Fusaria*, *Pythium ultimum*, and *Rhizoctonia solani*. U.S.R. No. 604 appeared to provide slightly less protection against these fungi, while Ceresan and Semesan Jr. were significantly inferior to both Arasan and Spergon.

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THE EFFECT OF ULTRAVIOLET RADIATION ON THE VIABILITY OF FUNGUS SPORES AND ON THE DEVELOPMENT OF DECAY IN SWEET CHERRIES

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The development of fungus decay in sweet cherries during their transportation and marketing constitutes one of the most serious problems that confront the sweet-cherry industry. A number of conditions contribute to the incidence of rot, and frequently the most important factor predisposing cherries to infection is the cracking of the skin by rains which occur as the fruit approaches maturity (11). Practices that are frequently employed to control decay in sweet cherries include (1) the pre-harvest application of fungicidal sprays or dusts, (2) careful handling to avoid unnecessary injuries, (3) sorting to remove damaged and infected fruit, (4) prompt storage under refrigeration, and (5) the use of carbon dioxide gas as a supplement to transit refrigeration. These methods are only partially effective, and the sweet-cherry industry is constantly looking for new and more effective means of reducing its losses from decay.

During the 1944 season a few packers of sweet cherries in the Pacific Northwest installed apparatus of the latest type for irradiating the fruit with ultraviolet light, as a means of controlling decay. In the absence of factual information either on the injuriousness of ultraviolet radiation to sweet cherries or on its effectiveness in controlling decay, a series of tests was conducted, the results of which are herein reported.

LITERATURE REVIEW

The lethality of certain wave lengths of ultraviolet light to bacteria and fungi has long been recognized, and in recent years attempts have been made to utilize radiation of this type for the commercial control of undesirable micro-organisms. Fulton and Coblenz (9), using a quartz mercury-tungsten lamp, tested the killing effect of ultraviolet radiation on the spores of 27 species of fungi and also determined its efficacy in the control of fungus decay in citrus fruits. Although the rays were able to destroy a high percentage of the spores on the surface of oranges, the final amounts of rot during a simulated marketing period were very much the same in the treated and the untreated lots.

In recent years improved ultraviolet germicidal lamps have been developed which restrict most of the radiation to a wave length of 2537 A.U. (3). Rays of this type are highly effective in the destruction of bacteria and fungi, and numerous applications of germicidal lamps for the commercial control of contamination by micro-organisms have been made. They have

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been employed in the baking industry (16), in cheese manufacturing and meat storage (8), and recently in the manufacture of penicillin (12). Garrett and Arnold (10) found that 2537 A.U. radiation offered promise in the sterilization of dairy equipment, and Kligman (14) pointed out its usefulness in the laboratory.

There apparently have been few successful applications of ultraviolet light for the control of bacteria and molds in fresh fruits and vegetables, although Ewell (8) has stated that "Losses in carload shipments of celery, etc., have been greatly reduced by sterilizing the surface water used for washing by similar (Sterilamp) ultraviolet radiation." De Ong (4) has suggested the use of these rays for the control of fruit decay, but no further reference to the application of ultraviolet light for the reduction of spoilage in fresh fruits and vegetables has been found.

The injurious effect of ultraviolet radiation on higher plants has been the subject of numerous investigations but no information on the sensitivity of cherries has been located. Fulton and Coblenz (9) reported that dosages of ultraviolet light lethal to a high percentage of spores of *Penicillium digitatum* caused no evident deleterious effects in oranges, and De Ong (4) observed no injury to apricots, plums, and berries treated with this radiation.

MATERIALS AND METHODS

The tests were conducted in a commercial sweet-cherry packing plant in which ultraviolet lamps had been installed for the express purpose of reducing decay. The apparatus was designed and installed so that, theoretically, the fruit would be freed of viable fungus spores, and thereafter, until packaged, would be protected from recontamination. A battery of eight 36-inch lamps was installed so as to irradiate a 12-foot section of the fruit conveyor. The first four lamps were 18 inches from the belt and the last four were 25 inches from it. Reflectors coated with aluminum paint directed the radiation downwards onto the fruit. The conveyor was constructed so that many of the cherries would be turned over when approximately halfway through the irradiated section, thereby enabling the light to contact directly most of the surface of the fruit. Under commercial operation the speed of the conveyor was adjusted so as to irradiate the cherries for 25 seconds. The belt extended forty-eight feet beyond the outlet of the battery of lamps so as to provide space for sorting the fruit. To lower the population of airborne spores in this section of the packing room, and thus to protect the fruit from recontamination, 12 lamps were installed approximately 7 feet from the floor on each side of the conveyor. They were equipped with reflectors to direct the rays away from the workers and the fruit.

The 30-watt lamps employed in the present installation are known commercially as "Uviare" germicidal lamps. Practically all of the ultraviolet light transmitted by them has a wave length of 2537 A.U. A complete discussion of the specifications and characteristics of these lamps is presented by Buttolph (3).

In a study of the lethality of ultraviolet radiation to fungus spores, the following seven fungi, all of which cause decay in sweet cherries, were employed: *Alternaria* sp., *Botrytis cinerea* Fr., *Cladosporium herbarum* (Fr.) Lk., *Penicillium expansum* Lk. emend. Thom., *Pullularia* sp., *Rhizopus* sp., and *Sclerotinia fructicola* (Wint.) Rehm. The spores of these organisms were washed with sterile distilled water from colonies that had developed on nutrient media (Bacto potato-dextrose agar) during an incubation period of three to four weeks at 65° to 80° F. In preparing the spore suspensions of *Alternaria* and *Penicillium*, a wetting agent (Wetsit Concentrate, at a dilution of 1 part to 1400 parts of water) was used with no evident inhibition of spore germination. However, when it was found that this material prevented germination of the conidia of *Sclerotinia fructicola* its use was discontinued. After the spore suspensions had been diluted to the desired concentration (approximately 200 spores per ml.) the surface of sterile agar plates was flooded as uniformly as possible with 1 ml. of the suspension. This is essentially the same technique as that employed by Fulton and Coblentz (9). That reliable results are obtainable by this method is shown by the work of Rentschler and Nagy (17), who found that the sensitivity to ultraviolet irradiation of bacteria in the air and on the surface of agar was the same. The seeded Petri dishes, with the lids removed, were exposed to ultraviolet radiation for periods varying from 30 seconds to 5 minutes. The lamps were 18 inches above the triplicate cultures employed for each treatment. The plates were incubated at 70°-74° F. and the number of colonies that developed in each was determined. Nonirradiated plates served as controls.

To determine the efficacy of a battery of lamps (installed 18 inches above the conveyor) in sterilizing the surface of the belt, the latter was lightly scraped after having been irradiated in a fixed position for 45 minutes. The scrapings were transferred to agar plates and the viability of the material was compared with that of similar transfers made from a portion of the conveyor that had not been exposed to ultraviolet light.

The effectiveness of the lamps installed above the sorting belt in lowering the population of viable air-borne spores was ascertained after the lamps had been in commercial operation the greater part of one day. Five Petri dishes containing sterile nutrient agar were placed on the sorting belt at approximately equal distances throughout its length. The plates were exposed for 5 minutes, and after a suitable incubation period the cultures were examined for the presence of fungus colonies. A comparable series of plates was exposed in a nonirradiated section of the room.

Two types of tests, one with artificially inoculated cherries and the other with naturally contaminated fruit, were conducted to determine the efficacy of the lamps in reducing decay. In the former test, sound Lambert cherries were disinfected by immersion in a 1:1000 solution of mercuric chloride, and then thoroughly rinsed in sterile tap water. After the stems had been pulled, the fruit was dipped in a suspension of spores of *Penicillium ex-*

pausum, dried, and divided into 4 lots (each consisting of three 100-fruit samples). The cherries were placed in a single layer on the conveyor belt and irradiated for 0, 0.5, 1, and 5 minutes. The conveyor was operated under simulated commercial conditions. The cherries were stored at 40° F. for 10 days and removed to 65° for 3 days (equivalent to the time normally required for shipments to reach New York City and for sale through retail channels); and they were then examined for stem-end infections. At both temperatures the relative humidity was maintained at 85 to 90 per cent.

In the tests with naturally contaminated cherries, 3 lots of fruit from different localities of north central Washington were used. The first lot consisted of Bing cherries grown near Malaga. This fruit was picked 4 days after a heavy rain and had a high percentage of skin cracks. Lot 2, from the Chelan district, comprised Lambert cherries with relatively little rain damage. Lot 3 consisted of the same variety from the Squillehuck Canyon near Wenatchee. This fruit was harvested 35 days after Lot 1 and showed no evidence of rain injury. The decayed and otherwise badly damaged cherries were removed from all lots, and the remaining fruit was typical of that being packed commercially. Each lot was thoroughly composited, stored at 50° F. for 24 to 36 hours, divided into the number of triplicate 3.5- to 5-pound samples requisite for the treatments, and exposed to ultraviolet radiation in the same manner as described for the artificially inoculated fruit. The samples, unless otherwise specified, were then stored 10 days at 40° with a relative humidity of 85 to 90 per cent. All lots were examined on removal from cold storage, and Lot 1 was inspected again after 2 days at 65°.

RESULTS

Viability of Spores Irradiated in Petri Dishes. The data in table 1 show that fungi differ markedly in resistance to the lethal action of ultraviolet radiation. Based on their reaction to this light, the organisms tested can be grouped into 3 rather distinct classes, namely, highly susceptible (*Penicillium expansum*), highly resistant (*Alternaria* sp.), and intermediate (the remaining five fungi tested). Approximately 92 per cent of the conidia of *P. expansum* were killed during an exposure of 30 seconds, and all were destroyed in 2½ minutes (Fig. 1). In the case of none of the other fungi was total destruction of the spores achieved, even during an irradiation period of 5 minutes.

A slight reduction in the number of viable spores of *Pullularia* occurred during an exposure of 30 seconds, but one or more minutes were necessary to cause a significant decrease in the germination of the spores of the 5 remaining molds. Slightly over half of the spores of *Pullularia* and *Sclerotinia* were killed in 2½ minutes, but a similar reduction in the viability of the conidia of *Cladosporium herbarum* required a treatment of 5 minutes. The spores of *Alternaria* were by far the most resistant, only a few being destroyed by the maximum exposure of 5 minutes.

The spores of *Cladosporium* and *Alternaria* are dark, whereas those of the other fungi tested are hyaline or relatively light. The resistance of dark-wall spores has been observed by other workers (9, 19), and Dimond and Duggar (6) suggest the possibility that susceptibility is influenced by such factors as pigmentation, spore size, and number of nuclei. When the

TABLE 1.—*The effect of ultraviolet radiation on the viability of the spores of several fungi that cause decay of sweet cherries*

Fungus	Irradiation period	Colonies per Petri dish	Spores killed*
	Minutes	Mean number	Per cent
<i>Alternaria</i> sp.	0.0	168.7 ± 4.91 ^b	0.0
	0.5	165.7 ± 7.88	1.8
	1.0	169.7 ± 6.01	0.0
	2.5	173.0 ± 9.61	0.0
	5.0	153.0 ± 3.00	* 9.3
<i>Botrytis cinerea</i>	0.0	84.7 ± 4.98	0.0
	0.5	76.7 ± 2.33	9.4
	1.0	78.5 ± 3.50	7.3
	2.5	72.5 ± 4.50	14.4
	5.0	25.3 ± 2.18	* 70.1
<i>Cladosporium herbarum</i>	0.0	230.7 ± 2.92	0.0
	0.5	218.0 ± 7.09	5.5
	1.0	220.3 ± 3.84	* 4.5
	2.5	174.3 ± 8.09	* 24.4
	5.0	104.0 ± 2.51	* 54.9
<i>Penicillium expansum</i>	0.0	134.0 ± 4.16	0.0
	0.5	11.0 ± 1.00	* 91.8
	1.0	3.3 ± 1.19	* 97.5
	2.5	0.0	* 100.0
	5.0	0.0	* 100.0
<i>Pullularia</i> sp.	0.0	252.0 ± 1.73	0.0
	0.5	189.7 ± 7.06	* 24.7
	1.0	179.7 ± 4.80	* 28.7
	2.5	105.0 ± 9.64	* 58.3
	5.0	75.0 ± 3.50	* 70.2
<i>Rhizopus</i> sp.	0.0	25.0 ± 2.08	0.0
	0.5	20.0 ± 1.52	20.0
	1.0	19.7 ± 2.19	21.2
	2.5	4.7 ± 1.19	* 81.2
	5.0	1.0 ± 0.58	* 96.0
<i>Sclerotinia fructicola</i> (<i>Monilia</i> stage)	0.0	89.7 ± 1.44	0.0
	0.5	87.0 ± 1.73	3.0
	1.0	89.3 ± 3.38	0.4
	2.5	38.3 ± 2.93	* 57.3
	5.0	12.0 ± 2.00	* 86.6

* Percentages marked with an asterisk differ significantly from the controls. Odds of significance 10:1 or greater.

^b Standard error.

poor penetration of the ultraviolet light, and the dark color and muriform structure of the conidia of *Alternaria* are taken into consideration, it is not at all surprising that this fungus proved so resistant to irradiation.

As previously stated, the installation of ultraviolet lamps and the commercial operation of the conveyor were such that the fruit would receive irradiation for only 25 seconds. Furthermore, the conveyor was designed

so that some of the cherries would be turned over after having traversed half the distance of the irradiated section, thereby exposing some parts of the fruit for only $12\frac{1}{2}$ seconds. The data in table 1 indicate that *Penicillium expansum* would be the only fungus appreciably affected by this treatment. However, since this organism is not the predominant cause of spoilage in sweet cherries, it appears doubtful that effective control of decay should be expected without greatly extending the period of irradiation.

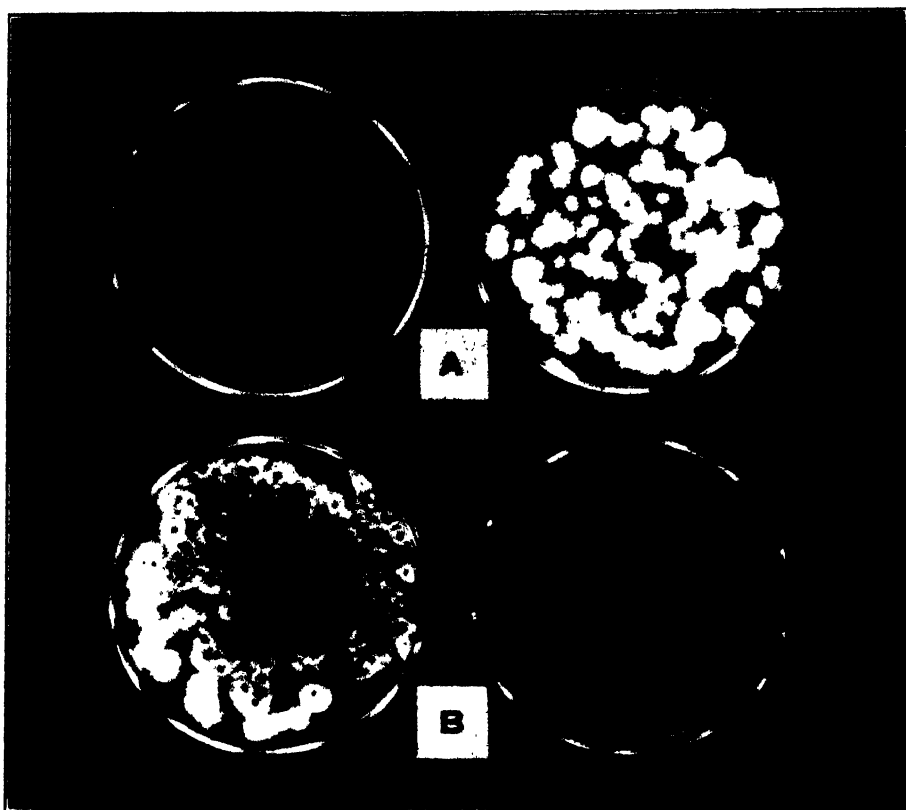


FIG. 1. Effect of ultraviolet radiation on 2 cherry molds. A, *Penicillium expansum*: plate at left irradiated $2\frac{1}{2}$ minutes, nonirradiated check at right. B, *Alternaria* sp.: plate at left irradiated 5 minutes; nonirradiated check at right. Note retardation of growth and pigmentation in treated culture of *Alternaria*.

During examination of the irradiated and control cultures, it was observed that colonies developing from spores that had been exposed to ultraviolet light were smaller and less pigmented than those produced by nonirradiated spores. This condition was first observed in cultures of *Alternaria* (Fig. 1), and at that time it was thought that the apparent inhibition of growth might have been due to a killing of the more exposed cells of the spore. However, when the same effect was observed in colonies formed by the other fungi, most of which had aseptate spores, this theory had to be abandoned. It was found that the amount of inhibition was directly related

to the period of irradiation. Also, the different fungi varied considerably in the degree to which they were retarded, the colonies of *Penicillium expansum* being considerably dwarfed by an exposure of 25 seconds, whereas those of *Cladosporium herbarum* required 2½ minutes for a noticeable reduction in rate of growth. In addition to the retardation of vegetative growth, there was a concurrent lag in the formation of reproductive bodies.

Delayed spore germination resulting from the action of ultraviolet light has been observed by Dimond and Duggar (5), and Rentschler, Nagy, and Mouromseff (18) have reported a retarded development of bacterial colonies following exposure to this type of irradiation. Other workers (1, 2, 15) have shown that 2537 A.U. radiation alters agar-water gels to such an extent that they are less suitable for the development of certain bacteria, the inhibition being due, at least in part, to an increase in acidity of the medium. It

TABLE 2.—The number of viable air-borne fungus spores on agar plates exposed in irradiated and nonirradiated sections of the packing rooms^a

Location of agar plates	Plate number	Colonies ^b per plate
Irradiated section—on sorting belt	1	13
	2	17
	3	23
	4	15
	5	22
	Average	18.0
Nonirradiated section—at elevation comparable to sorting belt	6	22
	7	19
	8	14
	9	21
	10	18
	Average	18.8

^a Spores allowed to settle on nutrient agar plates for 5 minutes.

^b The fungus genera obtained were principally *Penicillium*, some *Alternaria*, *Cladosporium*, *Oospora*, *Pullularia*, *Stemphylium*, and a few unidentified forms.

is not known whether the retardation of colony formation observed in the present study was due to the direct action of the light on the fungus or to some alteration of the substrate. Possibly both factors were operative.

Viability of Spores on Irradiated Fruit Conveyor. Material lightly scraped from 10 positions on the surface of a portion of the fruit conveyor that had not been exposed to ultraviolet radiation yielded fungus growth in each instance when transferred to nutrient agar. Similar material from a portion of the belt that had been irradiated continuously for 45 minutes produced colonies in 7 out of 10 transfers. The fungi isolated from the irradiated section included one or more species of *Alternaria*, *Penicillium*, *Pullularia*, and *Stemphylium*, all of which are known to cause decay of sweet cherries. The same organisms, with the addition of *Cladosporium* and *Mucor*, were obtained from the nonirradiated portion of the conveyor.

This test indicates that some of the fungus spores on the treated portion of the conveyor were destroyed, but it also shows that much viable inoculum

still remained. A quantitative test to determine the number of germinable spores on the irradiated and nonirradiated portions of the belt was not made, but judging from the data of table 1, it would seem that most of the exposed spores would be killed by treatment with ultraviolet light for 45 minutes. However, the mesh of the fabric belt, juice from injured cherries, dust, and other extraneous material would tend to protect some of the spores and thus reduce the effectiveness of the treatment.

Effect of Radiation on Air-borne Spores. The data in table 2 indicate that the ultraviolet lamps installed above the sorting belt did not reduce appreciably the number of viable fungus spores being deposited in this section of the packing room. Evidently the radiation did not contact the spores for the necessary lethal period. Also, inasmuch as the upper air in only about one-fourth of the room was irradiated, it is highly probable that spores from nonirradiated areas were deposited on the sorting belt. The conidia of *Penicillium*, which previously had shown little resistance to ultraviolet irradiation (Table 1), were found to be the predominant viable type settling

TABLE 3.—The effect of ultraviolet radiation on the development of blue-mold decay in artificially inoculated sweet cherries^a

Irradiation period		Decay	
Minutes	Av. wt. of 3 fruit samples Grams	Grams	Per cent
0.0 (Check)	975.7	852.7	87.4
0.5	929.0	849.3	91.4
1.0	910.0	817.0	89.8
5.0	919.0	836.3	91.0

^a Lambert cherries, with their stems removed, were dipped in a suspension of spores of *Penicillium expansum*, irradiated, and stored for 10 days at 40° F. plus 3 days at 65°.

on the sorting belt. It is obvious that the limited radiation employed would fail to protect cherries on the sorting belt from recontamination by air-borne spores; however, if the air of the entire room were irradiated some protection could logically be expected. Whether this protection would be of commercial significance has not been demonstrated in these experiments.

Blue-mold Decay in Inoculated Cherries Treated with Ultraviolet Light. As shown in table 3, it was impossible to obtain control of decay in injured cherries that had been artificially inoculated with the spores of *Penicillium expansum*. The maximum irradiation period of 5 minutes, which was 12 times as long as the commercial exposure, failed to effect any reduction in the amount of rot. It is recognized that conditions were extremely favorable for infection and for protection of the spores from the radiation; however, openings resulting from the accidental removal of stems constitute one of the important avenues of infection in sweet cherries. The spores of *P. expansum* have little resistance to ultraviolet light (Table 1), but under the conditions of this test—actually not different from severe natural conditions—the radiation in lethal dosage evidently failed to reach the spores. These results emphasize the fact, evidently largely overlooked by those pro-

moting the use of ultraviolet light for the control of fruit decay, that the destruction of exposed fungus spores in Petri dishes is a distinctly different problem from their destruction in favorable infection courts in plant tissue.

Effect of Irradiation and Storage Temperature on the Decay of Naturally Inoculated Cherries. In tests made with 3 lots of sweet cherry fruit that differed markedly in their susceptibility to infection, ultraviolet radiation was totally ineffective in reducing decay (Table 4). Negative results were obtained when cherries were exposed to the light for 10 minutes, a period

TABLE 4.—*The effect of ultraviolet irradiation and storage temperature on the decay of naturally inoculated sweet cherries*

Test fruit	Irradiation period	Storage		Mean decays ^a	
		Temperature	Period	At removal from storage	After 2 additional days at 65° F.
		F.	Days	Per cent	Per cent
Lot 1: Bing cherries severely damaged by rain	None	40	11	44.0 ± 1.74 ^b	54.9 ± 2.78 ^b
	25 seconds	40	11	46.9 ± 1.58	55.2 ± 2.30
	2.5 minutes	40	11	46.2 ± 0.80	53.3 ± 1.16
	5.0 minutes	40	11	41.7 ± 1.34	51.7 ± 2.06
	10.0 minutes	40	11	42.7 ± 2.07	52.4 ± 2.16
Lot 2: Lambert cherries very slightly damaged by rain	None	40	10	4.4 ± 0.55	
	None	50	5 }	7.2 ± 0.36	
	None	40	5 }		
	25 seconds	40	10	6.6 ± 1.50	
	5 minutes	40	10	5.1 ± 0.84	
Lot 3: Lambert cherries of relatively poor quality but without rain injury	None	40	10	21.0 ± 1.91	
	None	50	7 }	23.6 ± 0.95	
	None	40	3 }		
	25 seconds	40	10	19.0 ± 0.76	
	5 minutes	40	10	20.4 ± 0.54	

^a Percentages marked with an asterisk differ significantly from the checks. Odds of significance 19: 1 or greater.

^b Standard error.

24 times as long as that used commercially. Lot 1 had about the maximum amount of rain-damaged fruit that ordinarily would be tolerated in commercial handling, and the decay that developed in this lot emphasizes the hazard connected with the shipment to eastern markets of this class of cherries. The fruit of Lot 2 was of exceptionally fine quality, and had a cull grade of only 2 per cent when received from the grower. If the percentages of decay appear unduly high, it is because injured fruit with mycelium visible to the naked eye on the exposed tissue, but without evident penetration of the sound flesh, was classed as decayed.

The importance of proper lowering of transit temperature in the control of brown rot and blue-mold rot of sweet cherries has previously been discussed (7). The stimulatory effect of relatively high storage temperature

(50° F.) on the development of natural rots is shown in table 4 (lots 2 and 3). Irradiation of the fruit up to 24 times as long as is commercially practiced failed to reduce decay, whereas a difference in temperature of 10 degrees F. for only 5 days had a significant influence on the amount of spoilage. Ultraviolet radiation, as used in these studies, appears to offer little if any promise for the control of decay in sweet cherries, whereas the maintenance of proper storage and transit temperatures definitely reduces such losses.

One of the questions raised by some of the handlers of sweet cherries prior to the installation of ultraviolet-ray equipment was the possible injurious effect of this radiation on the fruit. Careful examination of the irradiated cherries and their stems failed to show any evidence of injury, even following the maximum periods of exposure. Similar results with oranges were obtained by Fulton and Coblenz (9).

DISCUSSION

It has been found that the spores of the more common fungi causing decay in sweet cherries can be destroyed by ultraviolet radiation if the requirements for proper wave length, intensity, and period of exposure are fulfilled. With information such as this at their disposal and with the knowledge that infection does not occur unless spores come in contact with fruit, some individuals have assumed that irradiation of contaminated plant material would largely prevent the development of decay. The fallacy of such reasoning has been shown in the present experiment by irradiating artificially and naturally contaminated cherries for periods considerably longer than necessary to kill most of the exposed fungus spores. It is the spores that have become lodged in ruptures in the skin of the fruit that are largely responsible for decay, and these are the ones that ultraviolet light seems incapable of reaching. Evidently most of the injury and contamination of the fruit occurs prior to the time it is unloaded at the packing plant, and the subsequent partial disinfecting of the cherries by irradiation has no appreciable effect on the development of decay.

Apples become heavily contaminated with fungus spores in the orchard (13) and there is little doubt that cherries are similarly inoculated. This condition is entirely different from that in bakeries and many other food-processing plants in which the original product is essentially free from spores or bacteria, and the problem is to protect it from contamination. Here the use of ultraviolet radiation to lower the population of spores in the air and on equipment would be expected to prove beneficial.

As a means of sterilizing the fruit conveyor, ultraviolet light proved partially effective, and if the upper air in the entire packing room had been continuously irradiated there is little doubt that the population of air-borne spores would have been reduced. However, from the results obtained in the present experiment it appears doubtful that either factor would appreciably affect the amount of decay developing in sweet cherries.

SUMMARY

The effect of ultraviolet radiation (wave length 2537 A.U.) on the spores of 7 fungi that cause decay of sweet cherries, and on the development of decay in this fruit was determined.

The fungi showed extreme variation in the susceptibility of their spores to the lethal action of the radiation. Most of the spores of *Penicillium expansum* were killed in 30 seconds, whereas only a few of the conidia of a species of *Alternaria* were destroyed after an exposure of 5 minutes. Spores with dark-color walls showed greater resistance than those with light-color walls. Colony formation from irradiated spores that were not killed was noticeably retarded.

The viable spore-load on the fruit conveyor in a commercial sweet-cherry packing plant was reduced by exposure to ultraviolet light, but the number of living air-borne spores in an irradiated section of the packing room did not differ significantly from the number found in a portion of the room that received no ultraviolet treatment.

Blue-mold decay in artificially injured and contaminated sweet cherries was not reduced by exposure to ultraviolet light.

Natural decay of sweet cherries was not controlled by ultraviolet radiation, even though the period of exposure was extended up to 24 times that employed commercially. As used in these studies, ultraviolet light appears to offer no promise for the control of decay, whereas the maintenance of proper storage and transit temperatures definitely reduces losses from decay.

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FIELD STRAINS OF TOBACCO-MOSAIC VIRUS

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In the laboratory many strains of the tobacco-mosaic virus may be isolated from a given field strain if the virus of the field strain is "plated out" on a necrotic-spotting variety of tobacco or certain other species of *Nicotiana*, and transfers are made from individual spots to susceptible plants (2, 3, 6, 7). These isolants may be less aggressive than the original field strain and be outgrown by the latter when both are mixed in a given plant. Some of these isolants might be purely laboratory strains which could not survive in the field. As a result, perhaps, of such studies as these, some laboratory workers not too familiar with the tobacco mosaic disease in the field, have taken the view that there is but one strain, or at best only a few strains, of the tobacco mosaic virus in the field. For example, one writer (1) states that by various isolation techniques, "literally hundreds of different strains can be obtained"; yet, he states, "when one goes into the field to obtain tobacco mosaic virus, one always finds the same strain in the tobacco fields. Why? The strain is nature's fittest." This not uncommon view has led to a great deal of confusion in the literature.

Several papers have been published, in which the virus used has been identified by the term Tobacco Virus 1 or *Nicotiana* Virus 1, inferring that this description is sufficient to identify the strain in the future. By definition Tobacco Virus 1 and *Nicotiana* Virus 1 include most of the field strains of the virus studied by virus workers from the time of Mayer to the present (5, 8). There is no way of knowing how many distinct strains of the tobacco mosaic virus have been studied during this period. When inoculations are made from cigars, cigarettes, or other dried tobacco, isolated yellow spots often develop on the first-invaded leaves, indicating that yellow strains are abundant in nature. Yellow and white strains are also frequently found in solanaceous weeds growing in old tobacco-growing areas. More conclusive proof that numerous strains are established in nature is furnished if collections are made from tobacco and solanaceous weeds in old tobacco-growing areas where tobacco is rotated with grass, thus allowing the perennial hosts to persist. Under these conditions the perennial solanaceous weeds become infected and act as reservoirs of virus for years.

Tobacco plants also become infected from dried mosaic tobacco of previous crops, from the hands of workers. Infection of seedling plants by individual virus particles may be frequent, and mutant strains, if present, could become established in pure culture in individual plants. If the strain happened to be nonaggressive it might, in the life of a vigorously growing tobacco plant, produce a more aggressive mutant and be replaced by it or it might become localized and lost. If aggressive, the new strain might be spread by the usual methods in the field and become a well-established field

strain with no chance of competition from less vigorous strains. In old tobacco-growing areas it is not unusual to find predominant strains on nearly every farm, strains which often differ from farm to farm. On large farms, where much transient labor is used, many strains of the virus are found. It is thus possible for numerous mutant strains to become established and maintain themselves in nature. There is accumulating evidence that numerous field strains of the tobacco mosaic virus occur. In 1928 Valleau and Johnson (10) mentioned 4 distinct field strains, in 1930 Johnson (4) described 7, and in 1936 Valleau (9) described 18 distinct field strains in a key which indicated the complexity of the tobacco mosaic problem in the field.

The purpose of this paper is to present additional evidence that numerous strains of the tobacco mosaic virus occur in nature and that these strains produce a complex of symptoms in tobacco, variable both with tobacco variety and environment, which makes it nearly impossible to describe a virus strain with sufficient clarity so that it can be identified with a strain described by another worker.

EXPERIMENTAL EVIDENCE

Four necrotic-spotting (N'N') tobacco varieties (11), Kelly and Judy (Burley), Kentucky 120 and 129 (dark fire-cured), in the greenhouse, were inoculated in duplicate with dried material of 54 field collections of tobacco mosaic virus. Inoculations were in triplicate, on field-grown plants of these varieties (except Ky. 120), with the 54 virus strains. Inoculations were also made to a non-necrotic-spotting variety (Ky. 16 n'n') but many of the viruses appeared so much alike on this variety that it was not considered a good test plant for showing differences in strains. The dried samples used in these tests were collected during the past 15 years from naturally affected tobacco plants from Kentucky fields, with the following exceptions: one each from tobacco from Florida and North Carolina; 3 from garden pepper, and 2 from *Physalis* sp. Included in these tests was a strain (No. 52 in table 1) of tobacco mosaic virus from the 1882 burley crop. Many more field strains of tobacco mosaic, apparently different from those used in this study, have been collected from tobacco plants and weeds, indicating that the number of tobacco-mosaic strains of natural occurrence is almost unlimited.

For convenience of description, the tobacco-mosaic strains are grouped in table 1, on the basis of symptoms caused in the necrotic-spotting varieties of tobacco. In the greenhouse the 54 tobacco-mosaic strains could be placed roughly in 19 groups; however, no two strains within a group were identical. Affected plants within a group had the same general type of symptoms but there were differences in color and distribution of mottling, degree of distortion, size and type of local spots, and degree of dwarfing, that were observable only by direct comparison.

In the field, symptom differences were greater and more varied. A minimum of 33 groups was required to classify them. For example, in the greenhouse, collection 9 caused slight dwarfing and distortion and produced yellow

ring-like patterns in all varieties tested, while in the field, the symptoms were almost identical with those caused by "pure white" mosaic (collection 1).

TABLE 1.—*Symptoms^a caused by field strains of tobacco mosaic virus in necrotic-spotting (N'N') varieties of tobacco*

Laboratory number of virus	Type of localization		Type of mottling		Degree of dwarfing		Degree of distortion		Type of necrosis		Group number	
	GH	F	GH	F	GH	F	GH	F	GH	F	GH	F
1	N	N	O	W	O	+	O	+++	O	O	1	1
40	N	N	Y	G	+	+	+	++	O	O	2	2
30	N	N	Y	G	+	+	+	++	O	O	2	3
51	N	N	Y	YR	++	+	+	+	O	O	3	4
7, 24	N	N	Y	YR	+++	+	+++	+++	St	O	4	5
10, 18	N	N	Y	Y	+++	+	+++	+++	St	O	4	6
32, 52	N	N	Y	G	+++	+	+++	+++	St	O	4	7
28	N	C	Y	G	+++	+	+++	+++	St	O	4	8
13, 14	C	C	YR	Y	+	+	+	++	O	O	5	9
29, 39	C	O	YR	Y	+	+	+	++	O	O	5	10
36	C	O	Y	G	+	+	+	+	O	S	6	11
43	C	O	Y	G	+	+	+	+	O	BS	7	12
38	C	O	G	G	+	+	+	+	O	B	8	13
16	C	C	Y	G	+++	+	+, E	+	O	B	9	14
22	C	C	YR	Y	+	+	+	+	B	S	10	15
49	C	O	Y	YR	+++	+	+, E	+	B	B	11	16
46	C	C	Y	Y	+	+	+, E	+	B	B	11	17
34	C	C	Y	Y	+++	+	+, E	+	B	O	11	18
9	O	N	YR	W	+	+	+	+	O	O	12	1
12, 15, 23, 33, 41	O	C	YR	Y	+	+	+	+	O	O	12	18
42	O	C	Y	G	+	+	+	+	O	O	13	8
4	O	O	G	YR	+	+	+	+	O	O	14	19
16	O	O	G	YR	+	+	+	+	O	O	14	20
19	O	C	G	Y	+	+	+	+	O	O	14	21
21, 26, 27, 53	O	O	G	YR	+	+	+	+	O	O	14	22
37	O	O	G	G	+	+	+	+	O	B	14	23
6	O	O	Y	Y	+	+	+	+	O	B	15	24
8, 11	O	O	Y	Y	+	+	+	+	O	BS	15	25
25	O	O	Y	Y	+	+	+	+	O	S	15	26
35	O	C	Y	Y	+	+	+	++	O	O	15	27
20	O	C	G	G	+	+	+	+	O	BS	16	28
45	O	C	G	G	+	+	+	+	O	S	16	29
2, 3	O	O	G	G	+++	+	+, E	+	O	O	17	30
5, 31, 54	O	C	G	Y	+++	+	+, E	+	O	S	17	31
44	O	C	Y	G	+	+	+	+	B	BS	18	28
48	O	O	Y	G	+	+	+, E	+	B	S	19	32
47	O	O	Y	G	+	++	+, E	+	B	BS	19	33
50	O	C	Y	G	+	++	+, E	+	B	BS	19	28

^a Symptoms in column GH occurred in the greenhouse, those in column F in the field. O indicates no symptoms.

N and C denote local necrotic or chlorotic spots.

Y, W, and G denote yellow, white, or green mottling.

YR denotes yellow rings.

B and BS denote burn and burn + necrotic speck spots in leaves.

S denotes necrotic speck spots in leaves.

St denotes necrotic streak of leaf veins and mid vein.

E denotes enation.

+, ++, and +++ denote slight, moderate, and severe distortion or dwarfing.

"Pure white" mosaic did not become systemic in greenhouse tobacco plants, but in the field this strain became systemic and caused marked distortion.

All viruses of greenhouse group 4 caused marked dwarfing and necrotic streaking of veins and midveins of invaded leaves. None of these caused streak in the field, in this test, and because of dissimilarity of symptoms, they were placed in 4 distinct groups. Other striking shifts in grouping in the field are observable in table 1. For example, the viruses of greenhouse group 14 formed 5 distinct groups in the field.

The descriptions in table 1 are not specific enough for identification of the viruses. They are given only to show that many strains of tobacco mosaic exist in nature. This study indicates that it is unsafe to assume that one is working with the same tobacco virus strain used by another worker merely because it was obtained from an infected plant in the field and compares fairly closely with published descriptions of symptoms on a single variety of tobacco and under one set of environmental conditions, particularly if the descriptions are based on the use of a non-necrotic-spotting variety (n'n') of tobacco. Two viruses may appear identical on a non-necrotic-spotting variety, whereas on a necrotic-spotting variety one may produce necrotic spots and streak, while the other may cause only mottle. The terms Tobacco Virus 1, common field strain, or wild strain of tobacco mosaic virus, if used to designate a specific virus commonly distributed in nature, are misleading.

SUMMARY

Fifty-four dried samples of tobacco, affected with tobacco mosaic, collected during 15 years in Kentucky tobacco fields and elsewhere, were used to inoculate necrotic-spotting varieties of *Nicotiana tabacum* in the greenhouse and field. The symptoms caused by these samples varied greatly both in the greenhouse and field. There were all shades of mottling from pure white to dark green, various degrees of distortion and dwarfing, various types and sizes of chlorotic and necrotic local spots, and various degrees of burn. No two collections caused identical symptoms, either in the greenhouse or field, although symptoms on replicate plants were identical. On a basis of similarity of symptoms and for convenience of arrangement in tabular form, the collections were placed in a minimum of 19 groups in the greenhouse. Thirty-three groups were the minimum into which the 54 strains could be placed in the field tests.

Most of these tobacco-mosaic-virus collections, if on n'n' tobacco plants could, by definition, be considered Tobacco Virus 1. For this reason it is suggested that the use of the term, Tobacco Virus 1, in the sense that it describes a specific entity, is not justified nor is it desirable to use the terms, common field tobacco-mosaic virus, or wild-type tobacco-mosaic virus to indicate a specific entity, for the same reason.

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LEAFHOPPER TRANSMISSION OF THE VIRUS CAUSING PIERCE'S DISEASE OF GRAPE AND DWARF OF ALFALFA

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This paper presents methods and data mentioned in previously published abstracts on the finding of leafhopper vectors of the virus causing alfalfa dwarf (6), the virus causing Pierce's disease of grape (3), and the association of the two diseases in the field (4). In addition, it describes the methods used in intertransmission experiments between alfalfa and grape, and the data obtained.

LEAFHOPPER TRANSMISSION OF THE ALFALFA DWARF VIRUS

Weimer (8, 9, 10, 11) determined that the alfalfa dwarf disease was caused by a virus transmissible by grafting, that it spread naturally in experimental plots in the field, and that it was apparently insect-transmitted. He (11) tested some insects, but did not report any transmission.

The distribution of diseased alfalfa plants in the fields follows, as Weimer remarks, a pattern indicative of insect transmission. During this investigation, in new fields planted adjacent to severely infested fields the disease could be found in the new plantings on the edge nearest the older plantings within 8 to 10 months after the new ones had become established. This occurred whether the new field immediately adjoined the old one or was separated from it by a road or by other crops so that each field was culturally treated as a separate unit. Virus-disease movement of this type points to an insect as the vector.

As mentioned in an abstract (6), the results of field caging trials in 1940 indicated that the virus was transmitted by insects. Two muslin-covered cages were constructed on land not previously seeded to alfalfa, with each cage covering 100 square feet. Alfalfa of the California Common variety, seeded under each cage, was allowed to grow for about three months. Then, collections of most insects commonly found in diseased fields were introduced into cage A, and insects were excluded from cage B as far as possible. Six months after the insects were introduced, 63 per cent of the plants in cage A had the dwarf disease as compared with 1 per cent in cage B from which insects were excluded. Five months later, less than 15 per cent of the original stand remained alive in the insect-infested cage A, and 44 per cent of these showed typical dwarf symptoms; in the insect-free cage, B, approximately 100 per cent of the original stand remained, and less than 2 per cent

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of these were diseased. The small percentage of disease in the insect-free cage B, was apparently the result of transmission by insects found to have accidentally gained entrance into the cage.

Transmission tests were made with over twenty species of insects, including aphids, beetles, leafhoppers, treehoppers, and thrips. These, except the thrips, were collected in diseased alfalfa fields and caged for 24 hours on dwarf-diseased plants before being caged on healthy plants. The thrips were collected on diseased alfalfa plants and transferred directly to healthy plants. Four months after the date of caging, the plants were dug and examined. The only transmissions occurred in two of the three trials with *Draculacephala minerva* Ball. A later trial with 100 individuals of this species, caged on dwarf-diseased alfalfa plants and subsequently transferred in one group to 30 healthy plants in a single cage, resulted in infection of

TABLE 1.—*Natural infectivity of four species of leafhoppers collected in their natural habitats and transmission of alfalfa-dwarf virus from diseased to healthy alfalfa plants by these leafhoppers*

Species of leafhopper	No. of collections	Lots of insects tested ^a		No. of plants inoculated	No. of transmissions
		No.	State of natural infectivity ^b		
<i>Draculacephala minerva</i>	15	2	Positive	57	55
		45	Negative	91	60
<i>Carniocephala fulgida</i>	5	0	Positive	0	0
		5	Negative	14	6
<i>Heliochara delta</i>	3	0	Positive	0	0
		10	Negative	26	12
<i>Neokolla circumlata</i>	5	14	Positive	93	69
		2	Negative	2	2

^a The number of hoppers in each lot tested varied from 20 to 40 individuals.

^b Positive indicates that these lots of leafhoppers were carrying the dwarf virus when collected; Negative, that they were not carrying the dwarf virus when collected.

93 per cent of the plants. A similar test with a closely related leafhopper, *Carniocephala fulgida* Nott., resulted in 19.4 per cent infection.

During 1941 through 1943, controlled insectary inoculations were made testing several species of insects collected in affected areas and in areas widely separated from any diseased alfalfa plantings. These collections were separated into lots of 20 to 40 individuals. Usually, before being used for transmission tests, each lot was fed on a series of healthy alfalfa plants to determine whether any insects in that lot were naturally viruliferous. After incubation of the plants used for these natural infectivity tests, it was possible to separate transmissions obtained by lots of leafhoppers proved naturally viruliferous from transmissions by those apparently nonviruliferous. As a rule, when the vectors were collected in areas where the virus was prevalent in alfalfa, they were naturally viruliferous. Collections in areas widely distant from dwarf-diseased alfalfa varied considerably in the percentage of lots proving to be naturally infective. Occasionally repeated collections

from an isolated population have been free of the virus. This method of obtaining nonviruliferous leafhoppers was necessary because of inability to rear sufficient populations in the insectary.

Table 1 shows the results of natural infectivity and dwarf-virus transmission tests with four species of leafhoppers. Because an attempt was made to collect the vectors in areas known to be relatively free of the virus, the majority of lots of three of the species were not naturally infective. The collections of *Neokolla* (*Cicadella*) *circellata* (Baker) were, however, usually so.

With *Draculacephala minerva* Ball a total of 47 lots, 45 of which were negative in the natural infectivity tests, were used in the transmission trials. After feeding on diseased alfalfa, the two lots shown to be naturally infective were fed on 57 healthy alfalfa plants, with resultant transmission of the virus to 55 of these. The naturally noninfective lots, after feeding on diseased alfalfa, infected 60 of 91 plants inoculated. All lots of *Carnucocephala fulgida* Nott. tested were naturally noninfective, and of 14 plants inoculated 6 were infected. With *Heliochara delta* Oman⁵ the ten lots tested were naturally noninfective, and 12 of 26 inoculated plants were infected. Fourteen of the 16 lots of *Neokolla circellata* (Baker) tested were naturally infective and infected 69 of the 93 plants inoculated. With the two noninfective lots, only two plants were inoculated; both developed the disease.

Numerous transmission trials were made with other collections for some of which the natural infectivity was not tested. The results are included in table 6.

LEAFHOPPER TRANSMISSION OF THE VIRUS CAUSING PIERCE'S DISEASE OF GRAPE

Pierce's disease of the grapevine (3, 5), recognized in California as early as 1884, was described by Newton B. Pierce in 1892 under the name "California vine disease" (7). It subsided around 1910, but became noticeably active again in grapevines about 1934 (5). In 1938 it was shown to be transmissible by grafting (2), and thereafter it was renamed Pierce's disease (3). The discovery that Pierce's disease was transmissible by grafting and was probably caused by a virus led to a search for possible means of spread. The pattern of diseased-grapevine distribution suggested some insect as a vector. The distribution followed two general patterns: first, diseased vines irregularly scattered through the vineyard; second, diseased vines concentrated in relatively small areas. Both types of distribution were found in most vineyard districts of the State on varied soil types and under different cultural practices. Localized spots of diseased vines were often found in portions of vineyards where water had been allowed to stand after irrigation and in other places where grasses continued to flourish, such as margins near irrigated pastures and alfalfa fields. These places usually supported numerous insects and the presence of diseased vines indicated that they included possible vectors.

⁵ Determined by P. W. Oman, Bureau of Entomology and Plant Quarantine, Division of Insect Identification, Washington, D. C.

Further evidence of insect transmission was obtained during 1940 in vineyards where insect light traps fitted with gas-discharge tubes and electrocuting grids had been installed. The traps were operated after dark for control of the grape leaf-folder. In a vineyard where an experiment with different light traps was being conducted,⁶ one 10-acre block of vines fitted with four 250-watt blue mercury-vapor-tube light traps had diseased grape vines around each trap, as shown in figure 1. Also, according to results of insect-net sweepings taken in this block of the vineyard, there was a higher population of several species of leafhoppers on the grass and weed

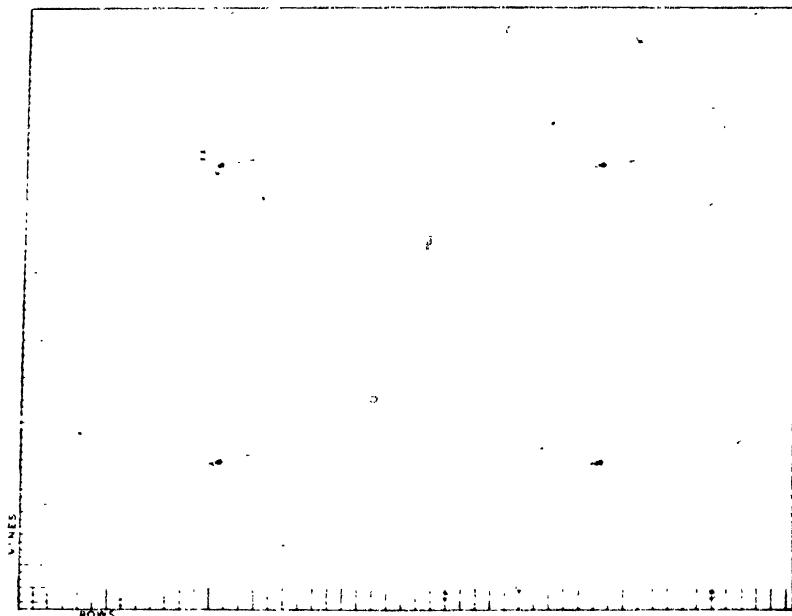


FIG. 1. Concentration of vines affected with Pierce's disease near the lights in a 10-acre vineyard in which were installed four 250-watt blue mercury-vapor-tube light traps fitted with electrocuting grids. The circles indicate the position of the diseased vines present in the vineyard in the spring of 1940. The arrows indicate the location of the light traps.

cover beneath the four light traps than elsewhere. The information obtained from these traps narrowed the scope of insects to be tested as possible vectors.

The general plan for discovering a vector of Pierce's disease was to test, wherever they could be found, all insects that might possibly transmit the virus. Preliminary tests were made on young vines growing in a plot located in a generally infested area. Large numbers of control vines were maintained, interspersed among the test vines to indicate possible natural spread. The insects were collected with hand nets, segregated, and placed in cheese-cloth bags with cellophane windows. These bags were placed over foliage of diseased vines, whence they were removed later, after 1 to 3 days' feeding of the insects. The bags containing the insects were then placed over foliage

⁶ This experiment with light traps was being conducted by J. K. Ellsworth, Division of Entomology and Parasitology, University of California.

of healthy test vines, where they were kept, usually, until the insects were dead.

During 1939, as previously mentioned (3), 54 species of insects were tested on 94 separate grapevines. Ten vines developed symptoms of Pierce's disease, but only one out of 215 controls did so. During 1940 a total of 60 species, including some of those previously tried, were similarly tested. Six out of 21 vines caged with *Draculacephala minerva* Ball and 3 vines out of 19 caged with *Carneoccephala fulgida* Nott. developed symptoms. Only 6 out of 506 control vines developed the disease. The symptoms were the same as on vines grafted with diseased wood.

During 1941 through 1943, tests were conducted in an insectary. All trials were made in insect-proof cages in a screen house or in a greenhouse.

TABLE 2.—*Natural infectivity of four species of leafhoppers collected in their natural habitats and transmission of Pierce's disease virus from diseased to healthy grape by these leafhoppers*

Species of leafhopper	No. of collections	Lots of insects tested ^a		No. of plants inoculated	No. of transmissions
		No.	State of natural infectivity ^b		
<i>Draculacephala minerva</i>	6	2	Positive	10	2
		23	Negative	40	16
<i>Carneoccephala fulgida</i>	3	0	Positive	0	0
		5	Negative	10	4
<i>Heliochara delta</i>	2	0	Positive	0	0
		4	Negative	11	7
<i>Nakolla circellata</i>	10	28	Positive	46	37
		8	Negative	8	4

^a The number of leafhoppers in each lot tested varied from 20 to 40 individuals.

^b Positive indicates that these lots of leafhoppers were carrying the virus of Pierce's disease when collected; Negative, that they were not carrying the virus of Pierce's disease when collected.

The grapevines used were rooted cuttings of the varieties Emperor, Malaga, Molinera, and Thompson Seedless, growing in greenhouse pots.

Leafhoppers from various locations were brought into the insectary and segregated into small lots. Many lots were first caged in succession on 2 to 3 healthy plants and held on each plant from 2 to 5 days to test them for natural carriers of the virus (state of natural infectivity). Each lot was then caged on a vine that had Pierce's disease and was held there 1 to 3 days. The lot of insects was then transferred in succession at 2- or 3-day intervals to one or more healthy vines for transmission trials. Table 2 gives the results of these trials with four species of leafhoppers: *Draculacephala minerva* Ball, *Carneoccephala fulgida* Nott., *Heliochara delta* Oman, *Nakolla circellata* (Baker). Leafhoppers in 2 out of 25 lots of *D. minerva* and 28 out of 36 lots of *N. circellata* carried the virus of Pierce's disease naturally when brought into the insectary for transmission tests. Previously noninfective lots of four species of the leafhoppers transmitted the virus from diseased to

healthy grapevines. Additional transmission tests with several lots of leafhoppers of the four species mentioned above were made without first testing the entire collection for natural carriers. The results of these tests, compiled with others, are presented in table 6. In addition to the above named leafhoppers, Frazier (1) recently reported 6 other species of leafhoppers as vectors of the Pierce's disease virus.

THE ASSOCIATION OF ALFALFA DWARF AND PIERCE'S DISEASE

The relation of alfalfa fields to the prevalence of Pierce's disease in grapes was noticed in infested districts where the two crops adjoined each other (4). Diseased vines were usually much more prevalent in the portion of vineyards nearest the alfalfa fields. In some instances, 100 per cent of the vines were infected in the first 4 to 6 rows adjacent to alfalfa. A typical

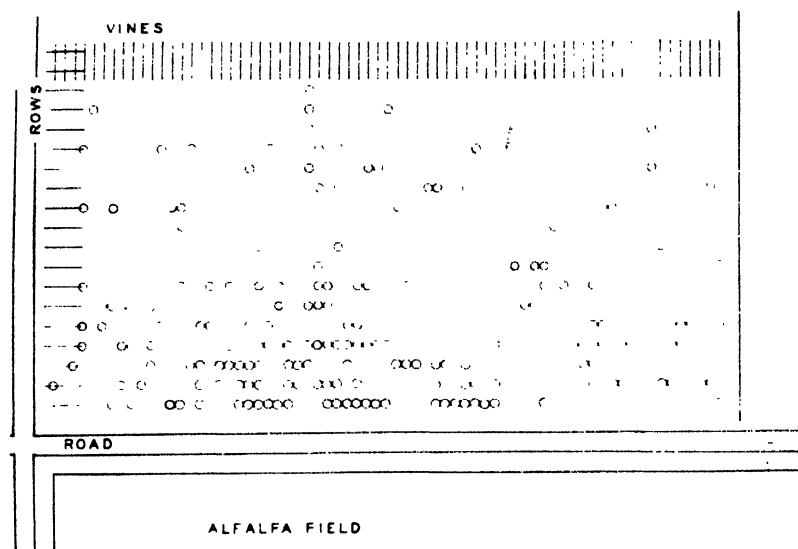


FIG. 2. Prevalence of vines manifesting Pierce's disease in a portion of a vineyard adjacent to an alfalfa field infested with dwarf. Very few diseased vines were found in the rest of the vineyard. Each circle indicates a diseased vine.

example is illustrated in figure 2, showing an alfalfa field, bordered on the north side by vineyard. The effect of the alfalfa was evident in the abundance of Pierce's disease in the first 12 rows of grapes adjacent to it.

The association suggested that alfalfa was harboring a vector of the grape disease, or that the alfalfa was an alternate host of the virus, or that it functioned in both capacities. Such alfalfa plantings usually contained a high percentage of dwarf-infected plants.

Furthermore, in vineyard districts where some alfalfa was planted and where Pierce's disease occurred, the alfalfa fields were usually infested with dwarf. The converse was also true. In certain districts the two crops existed separately, in some places with and in other places without their respective diseases.

The first efforts to connect the two diseases with the same causal agent were by means of mechanical inoculation. Neither dwarf of alfalfa nor Pierce's disease of grape was successfully transmitted by various means of juice inoculation. As previously reported, however, one can transmit dwarf to alfalfa and Pierce's disease to grape by grafting pieces of diseased plants on healthy plants. Pieces of roots from diseased alfalfa were therefore inserted into roots of healthy alfalfa and into stems of healthy grape; and, conversely, pieces of roots from diseased grape were inserted into roots of healthy alfalfa and healthy grape.

In these tests the following results were obtained: from diseased alfalfa to healthy alfalfa, dwarf was transmitted in 7 out of 10 trials; from diseased alfalfa to healthy grape there were no apparent transmissions; from diseased grape to healthy grape, Pierce's disease was transmitted in 37 out of 46 trials; and from diseased grape to healthy alfalfa, 12 out of 35 alfalfa plants showed dwarf symptoms after 6 months' incubation. This indicated the identity of the viruses.

INTERTRANSMISSION TESTS BETWEEN GRAPE AND ALFALFA PLANTS WITH LEAFHOPPER VECTORS

The discovery that dwarf of alfalfa and Pierce's disease of grape could be transmitted by the same vectors led to intertransmission experiments with the four species of leafhoppers. The plan of the following intertransmission experiments was to attempt to transfer the virus causing Pierce's disease of grape to alfalfa, and the virus causing dwarf of alfalfa to grape by means of the leafhoppers.

Leafhoppers of the species known to be vectors were collected from various natural populations, some from districts apparently free of either disease and others from districts known to be infested. The natural infectivity of some of these lots of leafhoppers used in intertransmission experiments was tested by caging a single lot of 20 to 50 leafhoppers on each of 2 to 4 healthy plants of both alfalfa and grape in succession. Each lot of insects was held on each individual plant for 1 to 3 days. The same individual lot was then caged on a diseased plant, either grape or alfalfa, and then subsequently transferred in successive order to healthy alfalfa and healthy grape plants or vice versa. The leafhoppers were retained on each plant from 1 to 6 days. The state of natural infectivity was not determined for all lots of field-collected leafhoppers. Only random sample lots of leafhoppers were taken from some lots and tested for the presence of natural carriers of the virus. Usually the remainder of the collection was divided into lots, and each lot caged directly on diseased plants, after which it was transferred to healthy ones. All test plants were then held in large screened cages or isolated in a greenhouse for incubation.

Some lots of field-collected leafhoppers proved to be naturally infective (viruliferous); that is, as collected in nature they contained individuals that carried a virus or viruses that would infect alfalfa and grape. The alfalfa

TABLE 3.—*Results of successive individual plant transfers of a single field collection of leafhoppers (N. circellata) which was naturally viruliferous when collected*

Plants on which leafhoppers were caged	No. of leafhoppers	No. of days on plant	Condition of plant after incubation
Alfalfa	25	3	Dwarf
Grape	23	2	Pierce's disease
Grape ^a	23	2	
Grape	10	2	Pierce's disease
Alfalfa	10	2	Dwarf
Grape	10	3	Pierce's disease
Alfalfa	8	2	Dwarf

^a Plant used as source of virus in experiment; however, incubation of the test plants showed the leafhoppers to be naturally viruliferous.

plants developed symptoms identical with dwarf, and the grape, symptoms identical with Pierce's disease. Table 3 shows the results of transmissions with such a lot of leafhoppers of *Neokolla circellata*.

Other lots of leafhoppers as collected from the field were apparently virus free as far as alfalfa and grape were concerned, for they did not transmit a virus to the healthy plants used in the natural infectivity tests, but did carry one to healthy plants after having been caged on diseased grape or alfalfa. Table 4 shows the results of intertransmission tests of the virus from dwarf-diseased alfalfa to healthy alfalfa and grape with a single lot of *Draculacephala minerva*. The 45 leafhoppers in this lot were first placed successively at two-day intervals on two healthy alfalfa plants and one healthy grape plant to test their natural infectivity. These three plants remained healthy showing that this lot of leafhoppers did not naturally carry the virus. After being fed on dwarf-diseased alfalfa, the insects were trans-

TABLE 4.—*Results of successive individual plant transfers of a single field collection of leafhoppers (Draculacephala minerva) from alfalfa to grape with dwarf diseased alfalfa as a source of virus*

Plants on which leafhoppers were successively caged	No. of leafhoppers	No. of days on plant	Condition of plant after incubation
Alfalfa	45	2	Healthy
Alfalfa	38	2	Healthy
Grape	34	2	Healthy
Alfalfa, dwarf ^a	34	2	
Grape	11	2	Pierce's disease
Alfalfa	9	2	Dwarf
Grape	9	2	Pierce's disease
Alfalfa	8	2	Dwarf
Grape	8	2	Pierce's disease
Alfalfa	8	3	Dwarf
Grape	8	2	Pierce's disease
Alfalfa	5	2	Dwarf
Grape	5	2	Pierce's disease
Alfalfa	5	2	Dwarf

^a Dwarf-diseased alfalfa plant used as virus source; the first three plants were used to determine the presence or absence of viruliferous individuals in the field-collected hoppers.

ferred alternately to 5 healthy grape and 5 healthy alfalfa plants. After several months all of the grapes manifested Pierce's disease, and all the alfalfa plants showed dwarf symptoms.

Table 5 shows the results of intertransmission of the virus from 5 differ-

TABLE 5.--Results of virus transmission tests from grape to alfalfa and alfalfa to grape with ten different field collections of leafhoppers (*Draeculacephala minerva*)

Leaf-hopper lot No.	Plant upon which collection was successively fed	No. of leaf-hoppers	Days on plant	Condition of plant after incubation
1	Grape ^a	15	3	Healthy
	Pierce's disease grape ^b	7	2	
	Grape	4	2	Pierce's disease
	Alfalfa	4	6	Dwarf
2	Grape	15	3	Healthy
	Pierce's disease grape ^b	11	2	
	Grape	8	2	Pierce's disease
	Alfalfa	4	6	Dwarf
3	Grape	15	3	Healthy
	Pierce's disease grape ^b	7	2	
	Grape	5	2	Pierce's disease
	Alfalfa	2	6	Dwarf
4	Grape	15	3	Healthy
	Pierce's disease grape ^b	12	2	
	Grape	11	2	Pierce's disease
	Alfalfa	6	6	Dwarf
5	Grape	15	3	Healthy
	Pierce's disease grape ^b	9	2	
	Grape	8	2	Pierce's disease
	Alfalfa	4	6	Dead (cause unknown)
6	Alfalfa	15	4	Healthy
	Alfalfa, dwarf ^b	14	2	
	Alfalfa	12	2	Dwarf
	Grape	10	6	Pierce's disease
7	Alfalfa	15	4	Healthy
	Alfalfa, dwarf ^b	13	2	
	Alfalfa	11	2	Dwarf
	Grape	11	6	Pierce's disease
8	Alfalfa	15	4	Healthy
	Alfalfa, dwarf ^b	15	2	
	Alfalfa	14	2	Dwarf
	Grape	14	6	Pierce's disease
9	Alfalfa	15	4	Healthy
	Alfalfa, dwarf ^b	12	2	
	Alfalfa	9	2	Dead (cause unknown)
	Grape	5	6	Pierce's disease
10	Alfalfa	15	4	Healthy
	Alfalfa, dwarf ^b	13	2	
	Alfalfa	13	2	Dwarf
	Grape	12	6	Pierce's disease

^a The first plant in each series was used to determine the natural infectivity of field-collected leafhoppers.

^b Plant used as a source of virus.

ent diseased grapes and from 5 different diseased alfalfa plants to healthy grapes and alfalfa with 10 separate lots of naturally noninfective leafhoppers made up from one field collection of *Draeculacephala minerva*. In this series the virus of Pierce's disease was transmitted from diseased grapes to healthy

grapes in 5 out of 5 trials, and to healthy alfalfa in 4 out of 5 trials. The virus from dwarf-diseased alfalfa was also transmitted to healthy grapes and alfalfa the same number of times. Two alfalfa plants listed in table 5 died before readings were made. Similar experiments were performed with the other species of leafhoppers previously mentioned.

It is further evident from data shown in tables 4 and 5 that the incubation period of the virus in the leafhopper, if such exists, is less than 4 days.

Table 6 summarizes the transmission of the Pierce's disease virus from diseased to healthy grape, and from diseased to healthy alfalfa, together with intertransmissions between grape and alfalfa. The results reported in this table include transmissions with naturally viruliferous and naturally non-

TABLE 6.—Transmission of the virus from diseased grape and alfalfa plants to healthy grape and alfalfa plants by four species of field-collected leafhoppers*

Species of leafhopper	Transmissions from diseased grape						Transmissions from diseased alfalfa					
	To healthy grape			To healthy alfalfa			To healthy grape			To healthy alfalfa		
	No. of plants		Per cent infection	No. of plants		Per cent infection	No. of plants		Per cent infection	No. of plants		Per cent infection
	Inoculated	Infected		Inoculated	Infected		Inoculated	Infected		Inoculated	Infected	
<i>Draeculacephala</i> <i>minerva</i>	118	67	56.7	98	50	51.0	115	78	67.8	504	442	87.7
<i>Carneccephala</i> <i>fulgida</i>	69	41	59.4	95	40	42.1	42	30	71.4	112	69	61.6
<i>Helochara</i> <i>delta</i>	61	34	55.7	75	37	49.3	73	42	57.5	115	71	61.7
<i>Neokolla</i> <i>circellata</i>	159	122	76.7	97	54	55.6	73	53	72.6	62	35	56.4

* A composite of results of tests made from 1939 through 1943.

viruliferous leafhoppers, conducted from 1939 through 1943. In all these tests, wherever transmission was obtained the grape manifested Pierce's disease, and the alfalfa showed symptoms typical of dwarf. The data show that only one virus is associated with Pierce's disease of grape and dwarf of alfalfa.

In completing these transmission studies, many collections of the four species of leafhoppers were made in areas thought least apt to contain naturally viruliferous populations. To run natural infectivity tests with each small lot of leafhopper vectors used would have consumed large numbers of plants. Therefore, in order to obtain a measure of the numbers carrying the virus, natural infectivity tests were made with only random samples from each field collection (Table 7). According to table 7 and also tables 1 and 2, the 3 species of leafhoppers *Draeculacephala minerva*, *Helochara delta*, and

Carneocephala fulgida did to some extent carry the virus naturally under field conditions. When, however, a comparison is made with the high percentages of transmissions obtained after feeding these leafhoppers on diseased plants, one can reasonably assume that the possible numbers of naturally viruliferous leafhoppers used in the transmissions reported added but little to the error in the results.

With the species *Neokolla circellata*, 59 per cent of the lots tested proved to contain naturally infective leafhoppers. This high percentage adds con-

TABLE 7.—Results of tests with random samples from collections of four species of leafhoppers made to determine whether certain populations used in transmission trials were naturally infective^a

Species of leafhopper	Total number of leaf-hoppers in all collections	No. of lots tested	No. of lots naturally infective	Per cent of lots naturally infective
<i>Draculacephala minerva</i>	6,650	38	5	13
<i>Carneocephala fulgida</i>	3,850	28	1	4
<i>Heliochara delta</i>	2,000	13	1	8
<i>Neokolla circellata</i>	3,085	80	47	59

^a Naturally infective means that the lot tested contained one or more viruliferous individuals when collected.

siderable error to the results obtained with transmissions from a known virus source with this species, except with lots previously proved not to be naturally infective.

SUMMARY AND CONCLUSIONS

The distribution and spread of alfalfa dwarf suggested transmission of the virus by insects.

Four species of leafhoppers—*Draculacephala minerva* Ball, *Carneocephala fulgida* Nott., *Heliochara delta* Oman, and *Neokolla circellata* (Baker)—were found that would transmit the alfalfa-dwarf virus from diseased to healthy plants.

The distribution of grapevines affected with Pierce's disease followed two general patterns: first, diseased vines irregularly scattered over the vineyard; second, diseased vines concentrated in small areas. Both types of distribution indicated that insects spread the virus.

The grouping of diseased vines under insect light traps added convincing evidence that the virus of Pierce's disease was transmitted by insects. Collections of insects made under the traps and compared with those made at some distance from the traps provided a valuable clue to the identity of the insects involved.

The same four species of leafhoppers that transmitted the alfalfa dwarf virus were at the same time proved capable of transmitting the virus of Pierce's disease.

Pierce's disease was usually more prevalent in vineyards of districts where considerable alfalfa was grown and in portions of vineyards adjacent

to alfalfa. Under such conditions the alfalfa fields were usually found infested with dwarf.

Root pieces from diseased grapevines inserted into the roots of alfalfa plants apparently transmitted the virus in 12 out of 35 trials, but root pieces from alfalfa similarly inserted in the stems of rooted grape cuttings did not transmit the virus to grapevines.

Naturally viruliferous field-collected leafhoppers of *Draeculacephala minerva*, *Carneoccephala fulgida*, *Helochara delta*, and *Neokolla circellata* transmitted to healthy alfalfa and grape plants a virus producing, respectively, dwarf and Pierce's disease.

The incubation period of the virus in the leafhopper *D. minerva* is less than 4 days.

Naturally nonviruliferous field-collected leafhoppers of the same four species transmitted the virus of Pierce's disease from diseased grapes to healthy alfalfa and grape and from diseased alfalfa to healthy alfalfa and grape.

The majority of field collections of *Draeculacephala minerva*, *Carneoccephala fulgida*, and *Helochara delta* were nonviruliferous, whereas 59 per cent of the lots of *Neokolla circellata* tested were naturally viruliferous.

According to the intertransmission experiments, the virus that causes Pierce's disease of grapes also causes dwarf of alfalfa.

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ELECTROPHORETIC STUDIES WITH THE PLANT VIRUSES¹

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Any scheme for the ultimate diagnosis of virus diseases in plants, or the identification of the viruses must, of necessity, involve the specific physical and chemical properties of the viruses themselves, in much the manner that the identification of bacteria involves their physiological behavior. Several means of testing for the presence of viruses in plants which have been suggested, other than that of testing the infectivity of the preparations, and which are based on the qualitative or quasi quantitative observations of the intensity of the action of oxidizing enzymes in plant extracts, on the determinations of quantities of ascorbic acid, glutathione, or plant pigments or similar products of plant metabolism in the plant sap, or on the accumulation and translocation of carbohydrates, or the nitrogen distribution in the plants, are of little value because these several tests are not specific for either the viruses or for the products of metabolism which are specific to any one of the virus diseases. In general, the color reactions with viruses and with proteins are so similar that differences in intensity of the reactions are extremely difficult to observe, and it is impossible by this means to distinguish one protein or virus from another. The picture relative to specific products of metabolism in the instances of the several viruses is obscure indeed, and attempts to correlate virus diseases with the diverse products of plant metabolism are entirely empirical.

There are, however, physical and chemical properties of the viruses and proteins—specifically, their electrokinetic properties—associated directly with the quality, quantity, and relative positions of the amino acids and other reactive units involved in virus and protein architecture, which may be made use of in diagnosis and identification. It is well known that colloidal particles will move through a fluid under the influence of an electric field. This migration is obtained by virtue of the electrostatic charges carried by the particles, and the charge in the surfaces of these particles is produced either through ionic adsorption or through ionization of the material making up the colloidal particle, or both. With proteins and with viruses the electrostatic charges in the surfaces of these entities are due to the ionization of the acidic and basic groups present, and the intensity of the net charge, resulting from the ionization, is related directly to the relative number of these active groups, as well as to the degree of their ionization, which in turn is a reflection of the architecture of the particles in question. The viruses and pro-

¹ This research has been terminated. The authors deeply regret that only an incomplete report may be made. Submitted December 17, 1944.

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teins are amphoteric, and the magnitude of the electrostatic charge carried by the particles or molecules is a function of the hydrogen ion activity in the solution in which they are dispersed; there is a specific pH-mobility behavior for each species of protein molecule or virus particle.

Given an aqueous protein solution placed in contact with the pure solvent in such a manner that there is a discontinuity in the physical properties at the interface, there will be generally, under electrophoresis, a migration of the region of discontinuity, or boundary, between the solution and solvent. The velocity of migration of the boundary will be characteristic of the protein in question, subject to the definition of conditions of pH, ionic concentration, temperature, viscosity of the solvent, and to the potential gradient. With a solution made up of two proteins there will be generally a boundary, or migration front, for each of the proteins. With a mixture of several proteins, there will be generally a boundary for each protein present in the mixture. These several migration fronts may be detected optically, and from such a study information may be obtained concerning the number of electrophoretically separable components in the mixture, the degree of electrical homogeneity of any component, and the concentration of any one of the components in the mixture. Where the components are separable, the pH-mobility behavior of each may be determined. It is also possible to isolate the individual components of such a mixture by electrophoresis.

The hypothesis which forms the basis of these studies is that with the extracts from plants infected with the viruses there will be specific migration fronts representative of the viruses involved, and that the viruses may be identified from their migration velocities under conditions defined with respect to pH, electrolyte concentration, and potential gradient.

METHODS

The electrophoretic experiments were carried out in a U-tube with a rectangular cross section, as designed by Tiselius (10) to effect maximum transfer to the bath of heat generated by the current passing through the U-tube. Analysis of the migration fronts was obtained by use of the adaptation by Lamm (4) and Toepler (11) of the device Foucault used as a means of accurately testing lenses for chromatic and spherical aberrations, together with the scanning camera designed by Longworth (5).

The optical scheme, as illustrated in figure 1, for the analysis of the migration fronts is based on the refraction of light passing through the interface between the solution and the solvent. The U-tube (10) in which the determinations were carried out is arranged in sections so that the filling may be effected leaving a very sharp interface between the solvent and solution, and no difficulty is experienced in observing initially the discontinuity in the physical properties at the interface. The interface becomes diffuse in time, due in part to diffusion. L is the source of illumination. S is a horizontal slit about 1 mm. wide. The lens, D, which in the instance of the apparatus assembled at Cornell University was 4.5 inches in diameter and

had a focal length of 23 inches, forms the image of the slit *S* at the point *P*. *E* is one arm of the electrophoresis cell, and the region designated as *A* is the region between the solution *m* (plant extract in our case) and the buffer *b*. *O* is the camera objective, having a focal length of 23 inches,⁴ and the camera is focused on the cell *E*. Under ordinary conditions all the light from the slit *S*, which is focused at the point *P*, enters the camera, and a clear image of the cell is formed on the photographic plate *C*. If there is a region in the cell where there is a continuous change in the index of refraction, as would be the case after the establishment of a concentration gradient, the light passing through that region will be refracted downward in a continuous manner through the width of the U-tube, because with each increment of distance traveled by the light beam through the region *A*, it enters a region of greater and greater refractive index. Consequently, the pencil of light passing through the region *A* will be focused at the point *P'* rather than at *P*. If the refraction is not too great, the light focused at *P'* will still enter the camera objective, and the image of the entire cell will be formed on the photographic plate. If, however, a knife edge *Q* is placed in a position im-

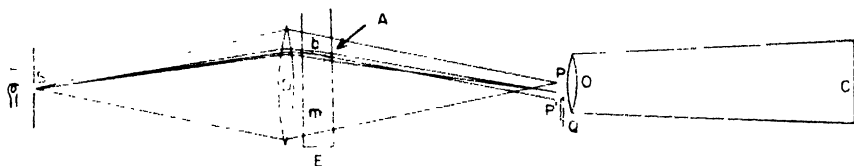


FIG. 1. Schematic diagram of the electrophoresis apparatus.

mediately below *P*, but above *P'*, the pencil of light focused at *P'* will not enter the camera objective, and there will be an unexposed area on the photographic plate corresponding to the region *A* in the electrophoresis cell. This will be true for each region in the cell where there is a variation of the index of refraction with height, that is, for each migration front.

The variation of the index of refraction with height in the region *A* is not abrupt if there has been a lapse of time. The curve obtained by plotting the index of refraction against the height of the cell will be S-shaped in the region of *A*, and the rate of change of refractive index with height will be a maximum in the center of the *S*. The pencil of light passing through this particular region will be refracted most, as indicated in figure 1. If the knife edge *Q* is moved vertically, the shadow on the photographic plate will first be only a thin line, corresponding to the center of the region *A*, but the shadow will widen as the knife edge approaches *P*; when the knife edge has reached *P*, the shadow will correspond in width to the entire region *A* in the electrophoresis cell.

The rear of the camera is arranged as indicated in figure 2, where *K* is a vertical slit about 1 mm. wide. One arm of the tube is brought to a focus on one slit, the other being masked, and the exposed area of the plate corre-

⁴ Approximate unit magnification of the cell is desired, and a long optical lever between the points *E* and *O* is desired in order to make the instrument sensitive to small changes in index of refraction in the cell.

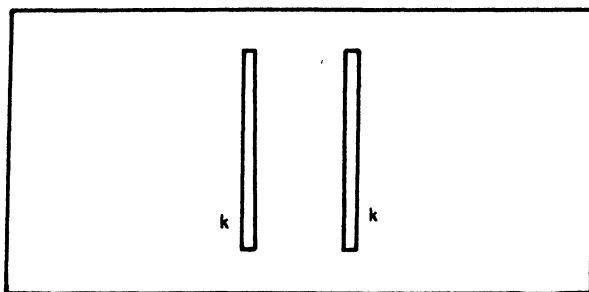


FIG. 2. Slits on the rear camera plate.

sponds to the area of the slit. Successive exposures of a plate at different positions along the rear of the camera, with the knife edge set progressively higher and higher with each exposure, results in a pattern of the type indicated in figure 3. Experimentally, the movement of the photographic plate is synchronized with the movement of the knife edge so that a continuous pattern is formed. These are the "scanning" patterns presented in this communication.

The concentration of each component in a mixture is proportional to the area of its shadow in the pattern on the photographic plate. It is also probable that there is a relation between the slenderness of the shadow, or peak, and the diffusion constant of the component.

Magnification of the image of the cell on the photographic plate was 1.3. The distance from the camera objective to the center of the cell was 42 inches, and from the center of the cell to the lens D was 11 inches. The photographic plate traveled 7.5 times faster than the knife edge Q.

The difficulty with electrophoresis of plant material has been in the preparation of the sample. Usually there is much pigment and suspended colloidal material in the extracts, which makes the photography difficult, and generally the concentrations of the components in the plant saps are too low for satisfactory scanning. It has been found useful in the preparation of the samples for electrophoresis to cytolyze the leaves with ether (1) initially. After cytolysis the vacuolar sap was pressed out with a hydraulic press and discarded. The vacuolar sap contains very little protein, and in tests with



FIG. 3. Scanning pattern built up by successive exposures after alteration of the height of the knife edge and the position of the plate holder.

plants infected with tobacco mosaic virus, it was found to contain little or no virus. The discarded sap does carry much pigment, however, as well as substrate for oxidizing enzymes. The pressed material was then washed with water several times, and after each washing the excess water was pressed out with the press. These washings do not affect the character of the scanning patterns obtained with the healthy tobacco plant, but they do effect the removal of additional pigment. In some determinations the pressed and washed material was ground in a Waring blender in the presence of buffer. In these instances concentration of the extractable proteins was obtained by reusing the same buffer with successive portions of leaf material, so that about 100 cc. of buffer were used in extracting 600 grams of leaves. Further concentration was obtained by placing the extract in cellophane tubing and hanging it before a fan. It has been found, however, that more efficient extraction may be obtained by grinding the pressed leaf material in a food chopper and extracting it subsequently with the buffer. An observation which has been of significance in these studies is that the intensity of pigmentation in the extracts, and the amount of suspended material that will not sediment in the centrifuge, is related to the age of the plant. Best results are obtained with young plants. With reference to the extracts alluded to above, they were centrifuged at 3,600 r.p.m. to remove plant debris, and then dialyzed against 2 liters of 0.1 M phosphate buffer at 4° C. for a minimum of 18 hours. This same buffer was used as the solvent *b*, as shown in figure 1. After the dialysis was complete, the extracts were centrifuged at 16,000–25,000 r.p.m. for 3–5 minutes, in order to remove plastids and other bodies, before electrophoresis determinations were made.

The potato tubers were ground in the Waring blender in the presence of 0.10 M phosphate buffer at pH 7.5, together with a trace of KCN, and then the slurry was centrifuged at about 3,000 r.p.m. for about 15 minutes. The supernatant liquid was added to an equal volume of a saturated solution of ammonium sulphate, and was again centrifuged. This second supernatant liquid was discarded, the precipitate was taken up in 0.10 M phosphate buffer, and dialyzed against the same buffer for about 18 hours. The solution was then centrifuged at 16,000–25,000 r.p.m. for 3–5 minutes before the electrophoresis determinations were made.

These preparations were never free from pigment and suspended matter, and it was necessary in most cases to resort to infra-red photography.

EXPERIMENTAL RESULTS

The scanning patterns for both the ascending and descending⁵ fronts in the U-tube for leaf extracts from healthy Turkish tobacco plants, at pH 7.5, are presented in figure 4, a and b. The time for electrophoresis was 30 and 60 minutes respectively. The indications are that there are only 3 protein

⁵ The plant sap is in the bottom of the U-tube. Since the solute migrates in the electric field, one boundary moves up and the other moves down. These migration fronts are the ascending and descending migration fronts. The arrows in each figure indicate the direction of migration.

components extracted by the methods used, although it is probable that there are actually three groups of proteins, none of which has been resolved electrophoretically under the conditions used. The velocity of migration (all velocities reported are for the descending boundaries) of component A was 0.83×10^{-4} cm./sec./volt/cm., that of component B was 2.1×10^{-4} cm./sec./volt/cm., and that of component C was 5.2×10^{-4} cm./sec./volt/cm.

The patterns with the healthy tobacco plant are not affected with the age of the plant up to at least three months.

The patterns obtained with tobacco plants five days after inoculation with tobacco mosaic virus, and at a time when no symptoms were evident, were identical with those shown in figure 4, a and b. Nine days after inoculation vein clearing was evident in the leaves, and the patterns obtained with leaves harvested at this time are presented in figure 4, C. These data are for 30 minutes of electrophoresis, and the buffer used was identical with that used for the healthy plants. Components A, B, and C present in the extracts from the nine-day-diseased plants were identified by their migration velocities as being identical with the corresponding components in the extracts from the healthy plants. Component V, migrating with a velocity of 3.9×10^{-4} cm./sec./volt/cm., is new, and it is believed to be the virus.

Marked symptoms were evident in the tobacco plants fifteen days after inoculation. The patterns obtained with these severely diseased plants are presented in figure 4, d. Time for electrophoresis was 30 minutes, and the same buffer was used as in the determinations previously discussed. Components A and B were present in the extracts, as evidenced from the shadows in the scanning patterns, but component V had become overwhelmingly predominant. The method of extraction precludes precise determination of the concentrations of the several components in either healthy or diseased plants, but after dealing with many samples the authors believe that there is no substantial change in the concentrations of the components A, B, and C with the development of the disease in the tobacco plants. The patterns shown in figure 4, d, were obtained with much more dilute extracts than those in either a, b, or c of figure 4, and in more concentrated preparations, evidence has been obtained that component C is also present.

The evidence that the normal protein components in the extracts are not materially altered by the development of the disease is supported by the data obtained by serological methods (8), since antibodies engendered in experimental animals with sap from tobacco plants infected with tobacco mosaic virus are precipitated by sap from healthy tobacco plants.

The reasons for believing that component V is the virus are three-fold, namely, the component is present only in the infected plants and increases in quantity as the disease progresses, the area of the shadow on the photographic plate caused by this component is more than thirteen times the combined areas induced by the other components in the extracts (9), and the slenderness of the peaks is suggestive of a substance with a small diffusion constant (2, 3, 7).

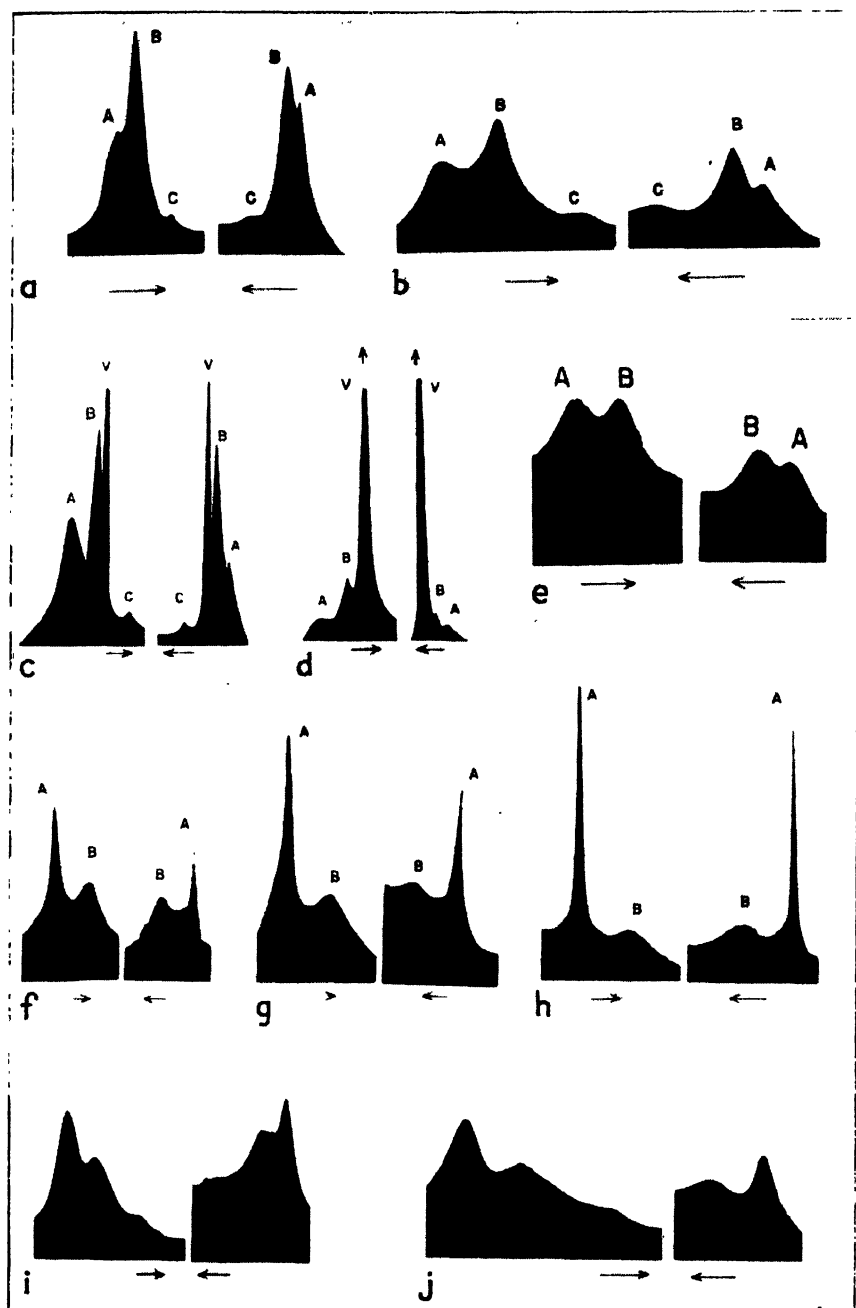


FIG. 4. Scanning patterns, descending at left, ascending at right. Healthy tobacco leaves with 30 min. electrophoresis (a) and with 60 min. electrophoresis (b). Tobacco leaves with tobacco mosaic virus nine days (c) and fifteen days (d) after inoculation. Healthy tobacco leaves (e) at pH 6.25. Tobacco leaves infected with the potato X virus at pH 7.5 (f), at pH 7.9 (g), and at pH 6.25 (h). Tobacco leaves infected with the potato Y virus, at pH 7.5, with 30 min. electrophoresis (i) and 60 min. electrophoresis (j).

The question may be raised regarding the completeness with which the virus may have invaded the plants after fifteen days, and the possibility may be suggested that the patterns shown in figure 4, d, were obtained with a mixture of diseased and healthy tissue. An attempt was made to answer this question. The severely diseased plants were cut back and two shoots from each were permitted to grow, as it was believed that in this manner leaf material might be developed under conditions where the opportunity for

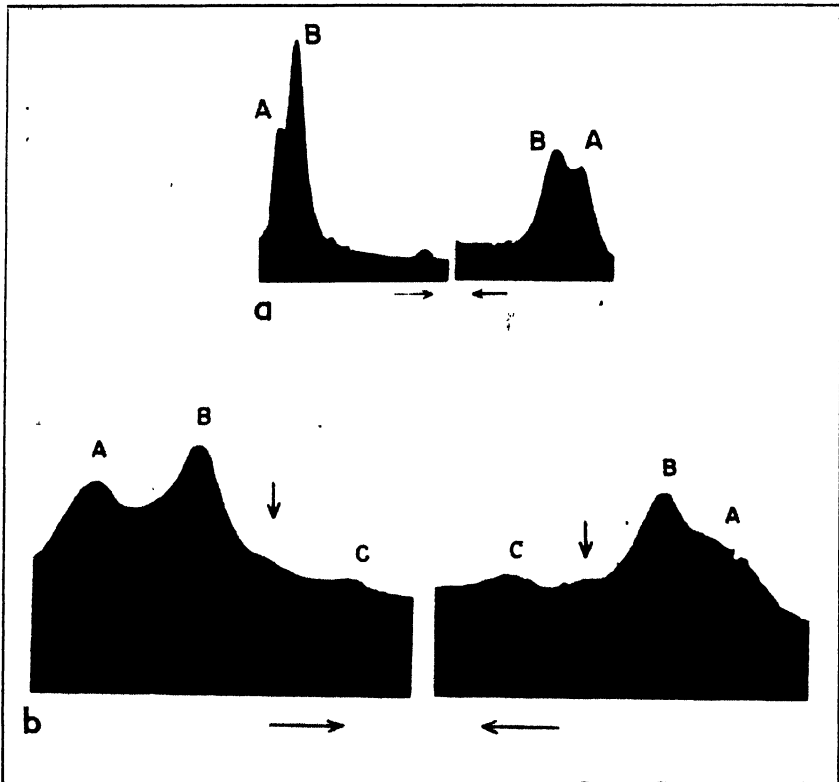


FIG. 5. Scanning patterns for tobacco leaves infected with Johnson's cucumber mosaic No. 1 (a) and with Price's indicator strain of cucumber mosaic No. 1 (b). Both are at pH 7.5.

systematic infection was maximum. These shoots were harvested after two weeks and the scanning patterns determined. They were identical with those presented in figure 4, d.

The abnormalities in the scanning patterns obtained with plants infected with other viruses are not so spectacular as those obtained with tobacco plants infected with tobacco mosaic virus. The patterns obtained with extracts from tobacco plants infected with potato X virus, which was obtained from potatoes, variety Green Mountain, are presented in figure 4, f. These data were obtained at pH 7.5. Time for electrophoresis was 60 minutes. In comparing these patterns with those obtained with the healthy plant, it

will be noted that the component A in these diseased plant extracts was present in a greater relative concentration than the corresponding component in the extract from the healthy plants. Its migration rate, at pH 7.5, was greater than that of the normal component A in figure 4, a and b. Component B, however, is the normal protein B in a and b of figure 4. The suspicion is that the normal component A and the X virus in the extracts are migrating with the same velocity under these conditions with respect to pH and electrolyte concentration. An attempt to separate, electrophoretically, the normal component A and the X virus by alteration of the pH of the buffer was without success. The patterns obtained at pH 7.9 are presented in figure 4, g, while those obtained at pH 6.25 are presented in figure 4, h. The patterns obtained with the healthy tobacco plant at pH 6.25 are presented in figure 4, e. A printable photograph for the patterns for the healthy plants at pH 7.9 is unavailable, but the patterns for the healthy plant as shown in a and b of figure 4 may serve as a control, since the +0.4 pH change caused but little alteration in the general shape of the pattern. The fate of component C had not been determined at the time the research was terminated.

The most reasonable interpretation of these data, with reference to component A, is that the virus has combined with the normal protein particles, imparting to them an altered electrophoretic mobility. This phenomenon is not uncommon with proteins (6).

The patterns obtained with extracts from tobacco plants infected with the potato Y mosaic are presented in figure 4, i and j. The time for electrophoresis was 30 and 60 minutes; the pH was 7.5. No opportunity was afforded for an attempt at separation of component A and the Y virus.

James Johnson's cucumber mosaic No. 1⁶ was transferred to tobacco plants and after symptoms were well developed the plants were harvested and the scanning patterns (figure 5, a) were determined with the extracts from the diseased leaves. Phosphate buffer was used, at pH 7.5. Components A and B are the normal tobacco-leaf proteins. The rapidly moving component apparent in the ascending arm of the U-tube has not been identified, and the fate of component C⁷ was not ascertained.

Price's indicator strain of cucumber mosaic No. 1⁶ was likewise transferred to tobacco plants, and the scanning patterns were determined with the diseased leaves, which were harvested after the symptoms in the plant were well developed. The data are presented in figure 5, b. Components A, B, and C are the normal tobacco-leaf proteins. The abnormal component at the head of the arrow migrated with a velocity of 4.0×10^{-4} cm./sec./volt cm.

The patterns obtained with leaf extracts with the healthy pea bean, variety Otenashi, are presented in figure 6, a, whereas the patterns obtained with extracts from bean leaves infected with Zaunmeyer's bean virus No. 4⁷ are presented in figure 6, b. Components A and B in the two scanning patterns migrated with velocities of -0.25×10^{-4} cm./sec./volt/cm. and $+1.9 \times 10^{-4}$

⁶ Kindly given to us by Dr. H. M. Munger.

⁷ Kindly given to us by Dr. B. L. Richards, Jr.

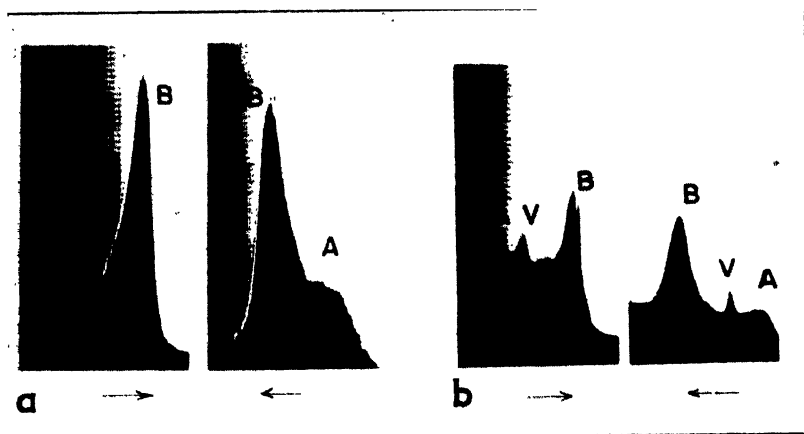


FIG. 6. Scanning patterns for healthy pea bean (a) and for pea bean leaves infected with Zaunmeyer's bean virus No. 4 (b). Both are at pH 7.5.

cm./sec., volt cm., respectively, and component V in figure 6, a, migrated with a velocity of 0.90×10^{-4} cm./sec./volt cm.

The patterns obtained at pH 7.5 with virus-free potato tubers are presented in figure 7, a. These patterns are not influenced by the time of storage of the tubers, and they are the same for all the virus-free tubers we worked with. Field-grown tubers present complications which have not been resolved, since most viruses with which they may become infected will remain in the stock, and these tubers, although they may appear healthy, frequently carry more than one virus. Patterns obtained with Green Mountain and Cobbler varieties of potatoes which were reputed to be healthy are presented in figure 7, b and c, respectively. The opportunity has not been available for the characterization of the two additional components which are obviously present in the extracts, but it is suspected that one of them is the X mosaic.

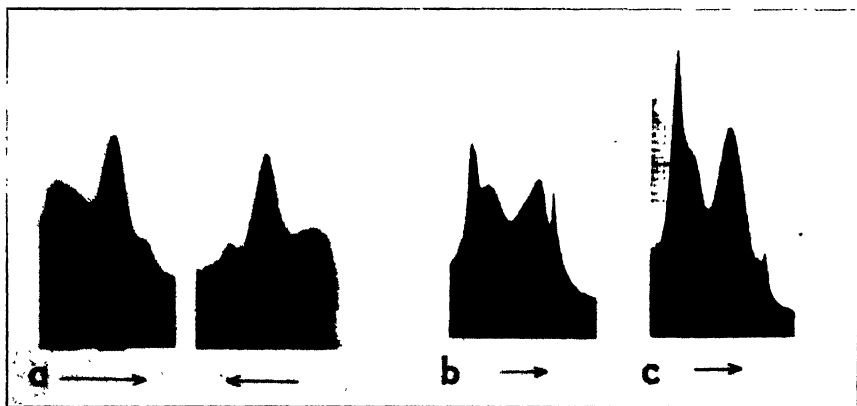


FIG. 7. Scanning patterns for healthy potato tubers (a), for field-grown tubers of Green Mountain potato (b), and for field-grown tubers of Cobbler potato (c). All are at pH 7.5.

DISCUSSION

Considered from the point of view of control of plant viruses, the initial concern should be, of course, with a general scheme for the diagnosis of virus diseases and the positive identification of the viruses. Ultimately, however, the control will be obtained through breeding programs based on a thorough understanding of the nature of resistance of plants to virus diseases and of the mechanism of immunity. There can be no question but that the key to that understanding will be in the dynamics of the leaf proteins, and it will be found through the employment of chemical science; the chemistry of the leaf proteins and plant protein metabolism are fundamental to the problem, and one cannot expect substantial progress in the control of these diseases by employing the empirical methods of the "practical" pathologist.

Our investigations had not reached the point where mixtures of viruses were involved when the investigation was terminated, but with each of the readily sap-transmissible viruses worked with, the patterns obtained are distinctive. In no case can it be said that the abnormal components in the several plant saps have been established as the respective virus components concerned, but the presumptive evidence that such is the case is strong. There is every reason to believe that the scheme used herewith may be used generally in diagnosis of virus diseases in plants and for the positive identification of viruses in infected plants. There are, however, technical difficulties which must be resolved.

The methods available for the extraction of leaf proteins are far from satisfactory, and the total quantity of protein extracted in any case is only a fraction of the total leaf protein. Those normal leaf proteins which have been extracted are difficult to handle; none of them have ever been crystallized as they do not lend themselves readily to manipulation. Concerning the methods of extraction, the use of ether for cytolysis appears to yield the best results for extraction in general, but, although ether appears to have no effect on the extractable proteins from the healthy tobacco plant, little is known regarding its effects on other plant proteins, nor is it known what influence it may have on the viruses. As has been indicated, the great virtue with the use of ether is in the elimination of much pigment, and, with the elimination of the vacuolar sap, a substantial concentration of the extractable proteins is obtained. The extraction of leaf proteins with solutions of electrolytes, with the exception of the dilute buffer solutions, has been neglected, and the same is true with respect to dilute acids and bases. The use of the Waring blender has no influence on the patterns obtained with the healthy tobacco plant, tobacco plants infected with tobacco mosaic virus, or with the virus-free potato tubers, but the possible influence of the blender on the surface denaturation of extractable proteins from other plants has not been ascertained.

These remarks regarding problems involved with reference to the leaf proteins apply to the viruses. Virtually nothing is known regarding the

physical and chemical properties of the viruses. With the exception of the few which have been isolated, very little may be said concerning the conditions under which they become soluble; their tendencies to become adsorbed on inert surfaces, such as cell debris produced during extraction; the ease with which they become denatured; their chemical reactivities or composition; their relative concentrations in the plant saps; and nothing is known regarding the rôle they may play in the production of disease.

The efforts to obtain scanning patterns have been limited in this study to a few of the viruses which are readily sap-transmissible. No attempt has been made to work with viruses which are not readily sap-transmissible or with the viriferous or nonviriferous insects known to be vectors of the viruses. No opportunity has been available for consideration of seeds infected with seed transmissible viruses.

Two suggestions are offered in conclusion. In the first place the pH-mobility curves of the viruses should be determined in so far as it is possible to do so—and many of these data may be obtained without the isolation of the viruses in question—as these data will be of considerable value in any sound scheme for the classification of the viruses, since the mobility behaviors are related specifically to their chemical composition and structure. The second suggestion comes from the observation that the patterns produced by plants belonging to the same genus have remarkably similar patterns. It would be worth while to determine the degree of similarity in the several genera.

SUMMARY

1. The moving boundary method of electrophoresis has been outlined.
2. Specific scanning patterns have been obtained for the extracts of healthy tobacco plants and for tobacco plants infected with tobacco-mosaic virus, with the potato X and potato Y viruses, with James Johnson's cucumber mosaic No. 1, and with Price's indicator strain of cucumber mosaic No. 1. These patterns are not identical.
3. Specific scanning patterns have been obtained for healthy pea beans, variety Otenashi, and for the pea bean infected with Zaumeyer's bean virus No. 4.
4. Extracts from healthy tobacco plants contain three proteins, and their concentrations and nature are not affected by the development of tobacco mosaic in the plant. The appearance of the abnormality in the scanning pattern obtained with tobacco plants infected with tobacco mosaic virus is correlated in time with the appearance of symptoms in the plant.
5. Two proteins are found in the extracts of the healthy pea bean.
6. The scanning patterns obtained with potato tubers (virus-free from virus-free seedlings) are the same from seedling to seedling, and are not influenced by the time of storage. Patterns from apparently healthy field-grown tubers have been observed to have more than one abnormality.

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TWO LEGUME VIRUSES TRANSMISSIBLE TO TOBACCO¹

E. M. JOHNSON

(Accepted for publication August 20, 1945)

Several virus diseases of alfalfa, *Medicago sativa* L., and white clover, *Trifolium repens* L., have been described and named.^{2, 3, 4, 5} Most of these have a rather wide host range among the legumes. Virus diseases of both plants occur in Kentucky, some of which may be similar to those described. Inoculation and physical-property studies of a virus disease of alfalfa and one of white clover, occurring in Kentucky, are described in this paper.

ALFALFA VIRUS

An occasional alfalfa plant with inconspicuous virus patterns has been seen in a few fields in Kentucky. Affected plants have a few irregular, often indistinct chlorotic blotches on an occasional otherwise normal leaflet (Fig. 1, B). The virus is mechanically transmissible to tobacco and several other species, with or without the aid of carborundum, if inoculations are made in the greenhouse to actively growing young plants during late fall or early spring but not during the summer or winter, possibly because of the effect of a higher temperature on virus content. The symptoms on various plants are described in table 1, and some are illustrated in figure 1, A, B, and D and figure 3, A. Physical properties of the virus are given in table 2.

The alfalfa virus is transmissible from alfalfa to alfalfa by aphids but, in these tests, not by mechanical methods even when carborundum was used. Aphids were collected on alfalfa in the field and placed on an affected alfalfa plant in the greenhouse. A few days later these were removed to 10 disease-free alfalfa seedlings. After several weeks all the alfalfa plants showed symptoms typical of the alfalfa mosaic. When inoculations were made to tobacco with this material, typical symptoms developed. Twelve healthy alfalfa seedlings were set out-of-doors in an isolated area on April 2. At the same time an alfalfa plant affected with alfalfa virus was set at each end of the row. On April 10, aphids were numerous on all plants. In November all the alfalfa plants showed typical symptoms of the alfalfa mosaic. Inoculations, made the following spring from these plants to tobacco, resulted in typical symptoms of alfalfa mosaic.

June Pink and Marglobe varieties of tomatoes, when inoculated with alfalfa virus, develop only small necrotic spots on rubbed leaflets. When, however, tomato cions are grafted on alfalfa-virus-affected tobacco, the cions

¹ The investigation reported in this paper is in connection with a project of The Kentucky Agricultural Experiment Station and is published by permission of the Director.

² Johnson, Folke. The complex nature of white clover mosaic. *Phytopath.* **31**: 103-116. 1941.

³ Pierce, W. H. Viruses of the bean. *Phytopath.* **24**: 87-115. 1934.

⁴ Weimer, J. L. Studies on alfalfa mosaic. *Phytopath.* **24**: 239-247. 1934.

⁵ Zaumeyer, W. J., and B. L. Wade. The relationship of certain legume viruses to bean. *Jour. Agr. Res. [U.S.]* **51**: 715-749. 1935.

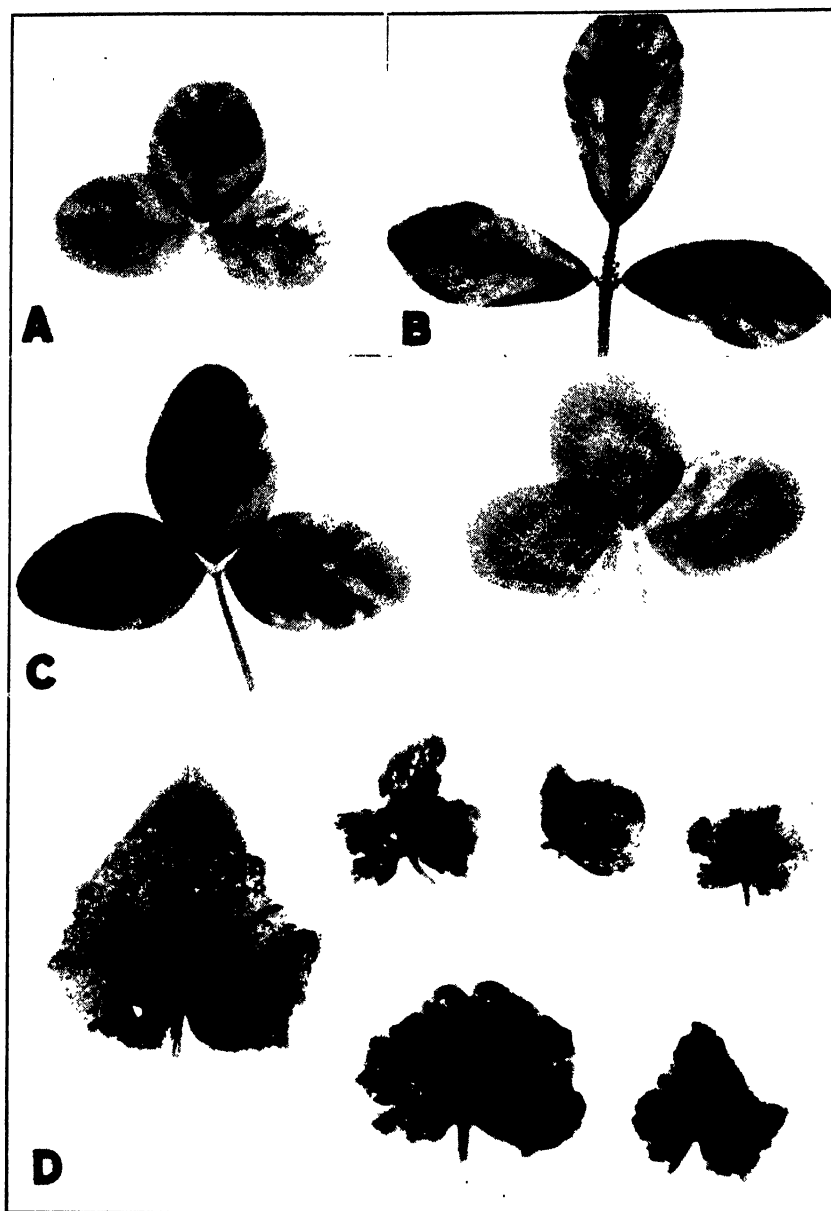


FIG. 1. A. Red-clover leaf affected with alfalfa virus. B. Alfalfa leaf showing typical mottling caused by alfalfa virus. C. Red-clover leaves affected with white-clover virus. D. Cucumber leaves showing mottling and distortion caused by alfalfa virus. All leaves from same plant.

show striking symptoms. Necrotic streaks develop on stems and petioles, the leaflets are small and crinkled and their margins are rolled downward. The leaves curve toward the stem giving the entire cion a dwarfed, bunched appearance.

Dodder (*Cuscuta sp.*), well established on a tobacco plant affected with alfalfa virus, was trained to a virus-free cucumber seedling. Four weeks later the cucumber plant developed symptoms typical of the virus (Fig. 1, D). Inoculations from this cucumber plant to tobacco resulted in symptoms typical of those produced by the alfalfa virus.

WHITE-CLOVER VIRUS

A virus disease of white clover has been seen for several years in small areas in an occasional pasture or lawn. Affected plants have irregular, pale yellow, mottled areas between the veins on a few to several, otherwise normal leaves. The virus is mechanically transmissible to tobacco and a few other plants (Table 1) if inoculations are made in late fall or early spring (Fig. 1, C, and Fig. 3, B).



FIG. 2. White-clover leaf affected with the white

Attempts to infect white-clover plants mechanically with the virus from affected white clover, red clover, tobacco, garden peas, and garden beans have been unsuccessful. The virus is transmissible to red clover from any of the species mechanically infected with the virus (Fig. 1, C'). No studies were made on dodder or aphid transmission of this virus.

COMPARISON OF ALFALFA VIRUS AND WHITE-CLOVER VIRUS

Although, in these studies, the alfalfa virus appears to have a wider host range than the white-clover virus the symptoms of both appear very much alike on plants susceptible to both viruses. This resemblance is sometimes striking on tobacco plants that have been infected for some time. On red clover and garden peas the symptoms are identical. The diseases resemble each other in that symptoms are masked at high temperatures. Neither virus can be transmitted mechanically, even with the aid of carborundum, when the temperature is about 80° F. or above. The physical properties are very much alike (Table 2).

Photographs and descriptions of the alfalfa virus on certain hosts were sent to Dr. W. J. Zaumeyer, who states: "The mottling of the alfalfa leaves

looks typical of the symptoms produced by the common alfalfa mosaic virus, but that means little since I feel that there are many strains of this virus.

TABLE 1.—*Means of transmission of alfalfa and white-clover viruses and the symptoms produced in various plants*

Host plant	Alfalfa virus		White-clover virus	
	Means of transmission	Symptoms	Means of transmission	Symptoms
Alfalfa, Grimm	Aphids, not mechanical	Systemic mottling	Not mechanical	
Red clover, Ky. 101	Rubbing	do	Rubbing	Systemic mottling
White clover	do	do	Not mechanical	Systemic mottling
Pea, Dwarf Telephone	do	Systemic mottling, Stem and petiole necrotic streaks, Dwarfing, Distortion	Rubbing	Systemic mottling, Stem and petiole necrotic streaks, Dwarfing, Distortion
Bean, Stringless Green Refugee	do	Necrotic spots on rubbed leaves	do	Systemic mottling, Necrotic spots on rubbed leaves
Tobacco, Ky. 16	do	Systemic mottling, Necrotic or chlorotic ring and line patterns, Dwarfing, Distortion	do	Systemic mottling, Dwarfing, Distortion, Necrotic or chlorotic line patterns
Tomato, June Pink and Marglobe	Rubbing, Grafting	Necrotic spots on rubbed leaves, Stem and petiole necrotic streaks, Dwarfing, Distortion	Not mechanical	
Zinnia, Pompon	Rubbing	Chlorotic or necrotic spots on rubbed leaves, Dwarfing, Distortion	do	
Cucumber, Long Green	Rubbing; Dodder	Systemic mottling, Chlorotic or necrotic spots on rubbed leaves, Dwarfing, Distortion	do	
Pokeweed, <i>Phytolacca americana</i>	Rubbing	Necrotic spots on rubbed leaves	do	
Pepper, California Wonder	do	Systemic mottling, Necrotic or chlorotic ring and line patterns, Dwarfing, Distortion	do	

. . . The virus which you have is apparently a strain of alfalfa mosaic and different from those which I have dealt with. The ones which I published

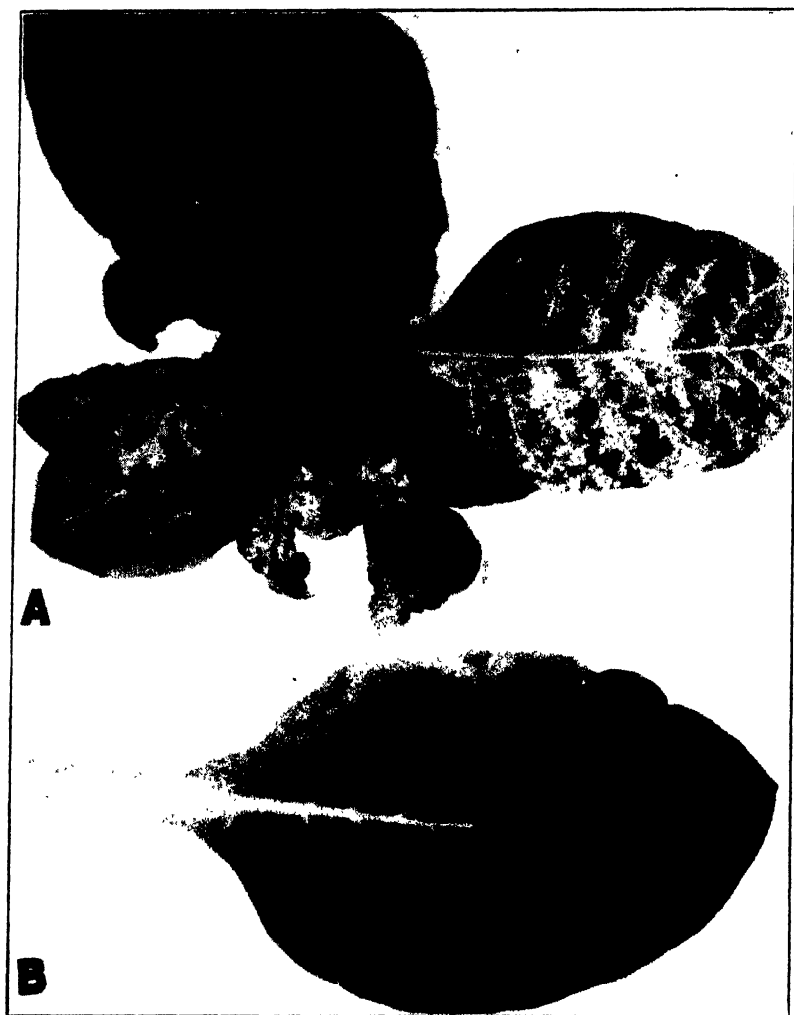


FIG. 3. A. Burley tobacco affected with alfalfa virus. B. Leaf of Burley tobacco affected with white-clover virus.

TABLE 2.—*Physical properties of alfalfa and white-clover viruses*

Physical properties	Alfalfa-virus infection		White-clover-virus infection	
	Positive	Negative	Positive	Negative
Tolerance to dilution	1: 100	1: 500	1: 100	1: 500
Thermal inactivation	58° C. (10 min.)	60° C. (10 min.)	55° C. (10 min.)	58° C. (10 min.)
Resistance to aging in vitro at room temperature	72 hrs.	96 hrs.	96 hrs.	120 hrs.
Resistance to drying at room temperature	15 days	30 days	15 days	30 days

on were not infectious to tomato, however, they did infect tobacco and cucumber but the symptoms on the latter were not as severe as yours."

The white-clover virus does not appear to be the same as F. Johnson's white-clover mosaic. Both viruses of his complex, pea mottle and pea wilt, have greater dilution values than the white-clover virus herein described and they affect neither tobacco nor tomato. From the brief studies reported in this paper, the alfalfa and white-clover viruses, tentatively, may be considered strains of alfalfa mosaic and related to the alfalfa mosaic virus described by Zaunmeyer and Wade.

SUMMARY

A virus disease of alfalfa and one of white clover occurring in fields are described. Although the two viruses have different host ranges they resemble each other in symptomology on tobacco, peas, and red clover. The physical properties appear very similar. Both appear to be strains of one virus, probably the alfalfa mosaic.

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CANKER OF HYBRID POPLAR CLONES IN THE UNITED STATES, CAUSED BY SEPTORIA MUSIVA

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(Accepted for publication September 1, 1945)

In the late summer of 1941, specimens of a branch and stem canker (Fig. 1, A) on clones of hybrid poplars, collected at a plantation of the Soil Conservation Service, Attica, New York, and at the New York State Nursery, Saratoga Springs, New York,² were referred to the writer for examination and a determination of the causal organism. The disease was reported to be prevalent in these experimental plantings on many of the clones of hybrid poplars that have been developed by Schreiner and Stout (10, 11) for pulpwood reforestation. The cankers did not resemble those characteristic of the common poplar diseases caused by *Dothichiza populea* Sacc. and Briard and *Cytospora chrysosperma* Fr., although mature pycnidia of *Cytospora* were present on or surrounding some of the cankered areas. Sporodochia of a species of *Fusarium* were conspicuous on a few of the cankers, but, in a superficial examination, no fungus could be found consistently in the diseased areas. On two of the cankers from the Attica collection a few small black pycnidia containing hyaline, curved, septate spores suggestive of a *Septoria* were present. The same fungus was obtained also in tissue cultures from a number of cankers in both collections. The *Septoria* corresponded with the description of *S. musiva* Pk. (8) that Bier (1) has recently reported as the cause of a serious canker disease of hybrid poplars in Canada. Cankers similar to those on the Attica specimens were recently collected³ from hybrid poplar clones planted by the Tennessee Valley Authority at Norris, Tennessee. No fruiting bodies of *S. musiva* were observed on the cankers, but tissue cultures developed typical pycnidia and spores.

Because of the apparent severity and importance of the disease on clones of hybrid poplars that have proved particularly adaptable for reforestation, the present paper gives a brief review of the hosts, the distribution of the disease, the symptoms, the causal fungus, and the results of inoculations to test the relative susceptibility of a few of the clones.

HOSTS AND DISTRIBUTION

A canker disease of Russian poplars (probably *Populus petrowskyana* Schneid.) grown in experimental plantings at the Northern Great Plains

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² These collections were received respectively from E. J. Schreiner, Senior Silviculturist, Northeastern Forest Experiment Station, Forest Service, U. S. Department of Agriculture, Philadelphia, Pa., and from J. R. Hansbrough, Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, New Haven, Conn.

³ The collection was made by G. F. Gravatt, Senior Pathologist, and the cultures were referred to the writer by R. W. Davidson, Pathologist, both of the Division of Forest Pathology, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland.

Field Station, Mandan, North Dakota, was reported by Johnson and Cobb (6) in 1923. The cause of the disease was unknown but it was described as "a disease which soon girdles the tree, generally near the base or at a crotch, and kills the portion above the canker." In 1936 the Northwest poplar, a rapid-growing hybrid, presumably balsam poplar (*P. tacamahaca* Mill.) × cottonwood (probably *P. deltoides* Marsh.), and considered to be native in North Dakota, was described by George (5) as "subject to canker." In Canada, the importance of canker on poplars in Saskatchewan has been emphasized (3, p. 28) and a brief study of the disease and its cause was made at Indian Head, Saskatchewan (4, pp. 24-26). An unidentified species of *Septoria* was isolated from leaf spots and cankers, and the susceptibility of *P. petrowskyana* and Northwest poplar was proved by wound inoculations of cuttings in the greenhouse.

A more detailed study of the disease at Indian Head and at the Petawawa Forest Experiment Station, Ontario, Canada, was reported by Bier in 1939 (1). He identified the causal fungus as *Septoria musiva*, which was described by Peck (8) in 1882 on living leaves of *Populus deltoides* (*P. monilifera* Ait.) at Albany, N. Y., and which is indigenous in North America. Bier's inoculations of cuttings in the greenhouse proved the susceptibility of the hybrid poplars *Populus rasumowskyana* Schneid., *P. petrowskyana*, and *P. berolinensis* Dipp. to stem infection. The Northwest poplar and another hybrid known as Saskatchewan poplar, with a similar parentage, also were susceptible. However, the native poplars *P. tacamahaca* and *P. deltoides* (designated by Bier as *P. balsamifera* L.), from which these hybrids were derived, proved to be resistant to canker formation. He found that leaf lesions were universally present on plantation trees susceptible to canker, and proved, by inoculations in the greenhouse, the susceptibility of the two species of native poplars, as well as the hybrid poplars, to leaf infection.

Thompson (12) studied *Septoria musiva* as the cause of leaf spot of poplars in the United States, and reported a twig lesion only on one shoot of *Populus szechuanica* Schneid. From his inoculations of poplar cuttings in the greenhouse, he found that 26 species and varieties are susceptible to leaf infection, including *P. maximowiczii* Henry and the Russian hybrids *P. petrowskyana* and *P. berolinensis*.

Recently a canker disease of poplars in Argentina was described by Sarasola (9) as caused by *Septoria musiva* and observed for the first time in that country in 1941. The origin of the fungus in South America is not yet known. Several hybrids and varieties of poplars, including clones developed by Schreiner and Stout (11), were reported as susceptible. The species *Populus laurifolia* Ledeb. and *P. simonii* Carr. (*P. przewalskii* Maxim.) also were reported (9) as susceptible. Leaf spots from which the same fungus was isolated occurred on the susceptible trees.

SYMPTOMS AND CAUSAL FUNGUS

The relation of leaf infection to the development of cankers on the hybrid poplar clones in the New York plantations has not yet been determined.

Bier (1) stated that spring infection of leaves and twigs of the current year's growth is brought about by ascospores of the perfect stage (*Mycosphaerella populorum* G. E. Thompson) produced in fallen leaves or in dead leaves that have remained over winter on affected twigs. This indicates the importance of leaf infection in the spread of the canker disease. Bier also found that, in the spring, mature perithecia and pycnidia with viable spores are sometimes present on cankers formed the previous year. Infection of newly developed leaves is evident by the presence of small, brown, circular or angular spots with yellowish or white centers. Several spots may eventually coalesce and increase in size until large areas are affected. Small black pycnidia appear in the lesions on both leaf surfaces. The leaves on the lower branches are most commonly affected, and early defoliation of these branches may occur. Infection of the twigs, as indicated by the specimens from the New York plantations, may take place through lenticels or wounds on the current season's growth. Bier (1) also reports infection through petioles and stipules. The bark of young lesions is dark brown or black and depressed, with a whitish central area in which small black pycnidia soon appear. On the most susceptible poplars the rapid spread of the fungus results in the girdling and death of the twig during the late summer. The fungus continues to grow from the axillary twigs into the main stem, on which perennial cankers may be formed. The presence of cankers around several leaf axils along the stem, with the swelling of the stem at the margins of the cankers and the constriction in the affected areas, results in a pronounced distortion of the stem. Pycnidia of *Septoria musiva* are rarely found on these cankers, but the dead bark and marginal callus tissue may be invaded by secondary fungi, particularly a species of *Cytospora*, probably *C. chrysosperma*. The continued development of the cankers eventually results in girdling and death of the tree. In the more resistant trees, the cankers develop more slowly and in some cases eventually become overgrown with callus tissue, with no further spread or girdling of the stem.

Bier (1) and Thompson (12) give brief discussions of the taxonomy and morphology of *S. musiva*, and the characteristics of the fungus in the present study, on the hosts and in culture, agree with their descriptions. The spore measurements for conidia are as follows: From pycnidia on leaves—Bier's measurements (330 spores from 4 hosts) $17.2-51.6 \cdot 3-4 \mu$, Thompson's measurements (850 spores from 7 hosts) $28-54 \cdot 3.5-4 \mu$. From pycnidia on cankers—Bier's measurements (180 spores from 3 hosts) $21.5-55.6 \cdot 3-4 \mu$, the present study (200 spores from 2 clones) $24-56 \cdot 3.5-4 \mu$. Sarasola (9) gives measurements of 1200 spores from pycnidia on the leaves of various hosts as $18.5-70 \times 2.0-4.9 \mu$. This latter range is indicative of the wide variation in spore length mentioned by Davis (2), who considered *S. musiva* as a composite species. Thompson (12) described *S. populicola* Pk. as distinct from *S. musiva*, having a range in spore length of $45-80 \mu$, with an average of 62μ . The average of spore length reported by Sarasola was 43.4μ , which would seem to place his fungus in *S. musiva*.

In the present study the causal fungus was isolated and grown on Leonian's medium (7), from single spores produced in pycnidia on the cankers in the Attica collection and from the inner bark of nonfruiting cankers of both collections. The colonies grew slowly and produced pycnidia and spores during a comparatively short period, about 2 or 3 months. The entire surface of the colony then became overgrown with a profuse white or pinkish mycelium and no further fruiting took place. This mycelium grew readily in transfers, without the production of pycnidia or spores. Transfers by means of single spores from fruiting cultures produced mature pycnidia for some time, but eventually, after about 8 or 10 months, transfers of this type also failed to sporulate. Repeated attempts to obtain fruiting pycnidia by transfers of the mycelium to sterilized poplar twigs failed. However, when the mycelium from the Attica isolations was inoculated into living twigs of susceptible trees, cankers and typical fruiting bodies were formed. The fruiting period of the isolations from the Saratoga specimens was shorter than that from the Attica specimens and in inoculations the mycelium failed to produce fruiting bodies on the cankers. Bier (1) and Thompson (12) found that in culture some colonies never form pycnidia and spores.

INOCULATIONS

Three series of inoculations were made on cuttings from 10 clones of hybrid poplars selected by Schreiner for their vigorous growth and for their apparent variation in susceptibility to the disease in the nursery. The parentage of the clones is as follows:

OP-1	<i>Populus nigra</i> L.	<i>P. laurifolia</i> Ledeb.
OP-2	do	do
OP-4	do	do
OP-11	do	<i>P. trichocarpa</i> Hook.
OP-12	do	var. <i>betulifolia</i> (Pursh) Torr. <i>P. trichocarpa</i>
OP-41	<i>P. maximowiczii</i> Henry	<i>P. trichocarpa</i>
OP-46	do	<i>P. berolinensis</i> Dipp.
OP-47	do	do
OP-52	do	<i>P. nigra</i> var. <i>plantierensis</i> (Simon-Louis) Schneid.
OP-55	<i>P. candicans</i> Ait.	<i>P. berolinensis</i>

The cuttings were rooted and potted in the greenhouse, and the current season's growth was inoculated in June. The inoculum consisted of pieces of sporulating pycnidia from the single-spore cultures from the Attica specimens and from tissue cultures from the Saratoga specimens. Ten inoculations (1 on each clone) were made by placing the inoculum from the Attica isolate in uninjured leaf axils. In 10 additional inoculations with the Attica isolate the inoculum was placed in contact with the exposed wood of small triangular stem wounds made with a sterile scalpel. Two similar series of inoculations were made with inoculum from the Saratoga isolate. The inoculated areas in all cases were protected by wrappings of wet cotton and waxed paper tied at both ends, the wrappings being removed after 10 days. A control, consisting of a stem wound without the insertion of the inoculum, was made for each pair of wound inoculations.

From the wound inoculations with inoculum from the Attica isolate positive results were obtained on clones OP-2, 46, 47, and 52. Typical cankers and mature pycnidia were formed, and *Septoria musiva* was reisolated in single-spore cultures in September. On clones OP-4, 11, 12, and 41 open wounds resulted, but no fruiting bodies of *S. musiva* were present and the fungus failed to develop in tissue cultures from the wounds. This may have been due to the fact that other fungi, such as *Fusarium* and *Cytospora*, were abundant and may have overgrown the more slowly developing *Septoria*. The wounds on OP-1 and 55 healed normally, as did those on all the controls. On clone OP-4 infection occurred through a leaf petiole from the inoculum placed in the leaf axil, and *S. musiva* was reisolated from spores in pycnidia on a small stem canker.

The inoculations with the Saratoga isolate produced partially healed wounds on OP-2, 11, 41, 46, 47, 52, and 55, with no evidence of pycnidia of *Septoria musiva* and the fungus failed to develop in tissue cultures from the wounds. On clones OP-1 and 12 the wounds inoculated with this isolate healed over in the same manner as the controls. Because of the failure to obtain positive results with this isolate it was not used in the two following series of inoculations.

The uninoculated cuttings remained in an unheated greenhouse during the winter and were planted outdoors the following spring. Two series of inoculations were made in June, one, on the stems of the previous year's growth, consisting of the application of mycelium to the exposed wood of stem wounds as in the preceding experiment, and the other, on the current season's growth, of a spore suspension on uninjured leaves and petioles, and on stem wounds. The wound inoculations were protected with wet cotton and waxed paper wrappings for 10 days in the first series and for 72 hours in the second series. The inoculum was derived from single-spore cultures from the Attica isolate. In the first series 2 stem inoculations were made on each of the 10 clones. In both series, controls were made on each clone to correspond with the different types of inoculations.

The cankers resulting from the inoculations in the first outdoor series were collected in September for examination and reisolation of the fungus. *Septoria musiva* was reisolated in single-spore cultures from the two cankers on OP-2 and from one canker on OP-46, 47, and 52 (Fig. 1, B, C). Fruiting bodies of a species of *Fusarium* were present on the second canker on each of these three latter clones, and no other fungus was obtained from tissue cultures. The two cankers on OP-12 and 41, and one on OP-4 were overgrown with a species of *Cytospora* and of *Phomopsis*. The second canker on OP-4 and the two cankers on OP-11 and 55 had healed.

The cankers in the second outdoor series, resulting from infection of stem wounds, were collected and the fungus reisolated one month after inoculation. At this time reisolations were made also from the leaf spots and petiole infections. In this series no secondary fungi had invaded the inoculated stem wounds and mature pycnidia of *Septoria musiva* were present, except

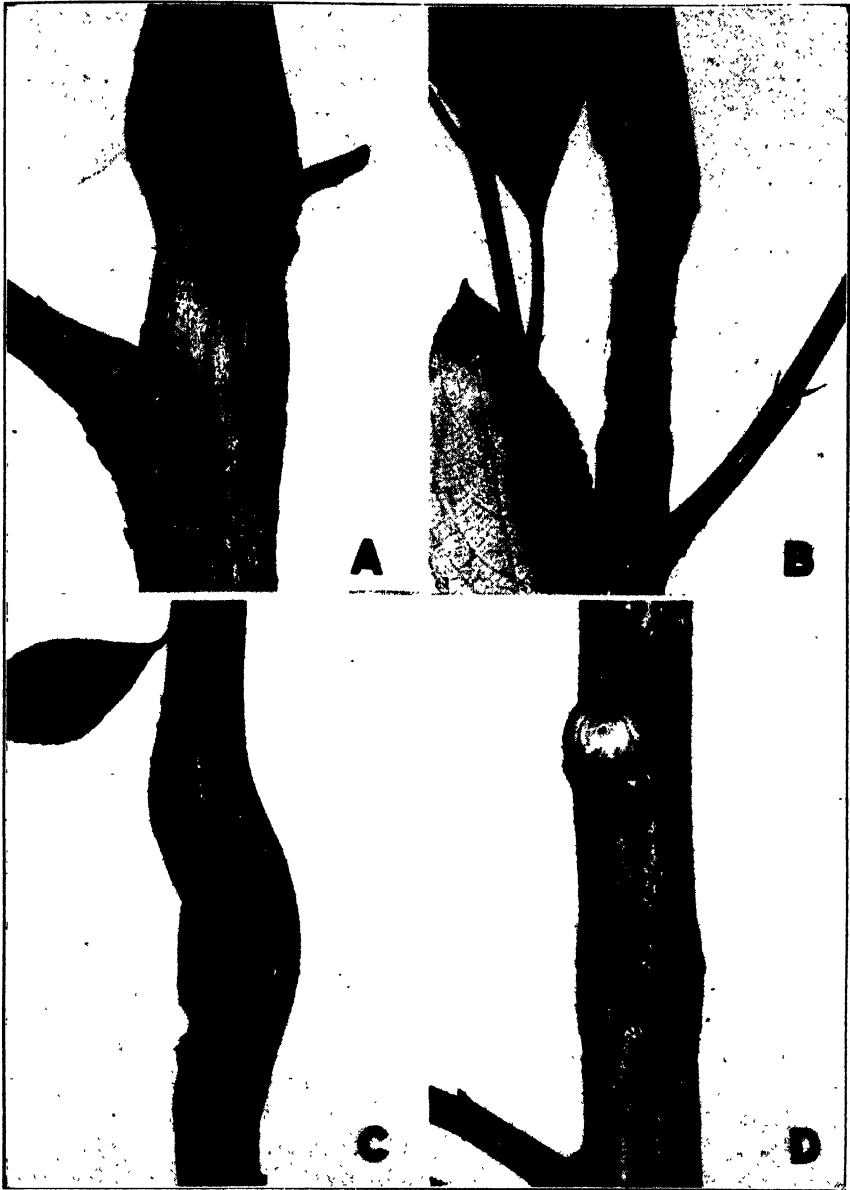


FIG. 1. Cankers caused by *Septoria musiva* on clones of hybrid poplars. A. Canker on a 3 year-old stem of a plantation tree. $\times 1$. B. Girdling canker from stem wound inoculation on clone OP-2. $\times 1$. C. Canker from stem wound inoculation on clone OP-46. $\times 1$. D. Stem wound in inoculation control on clone OP-4, showing normal healing. $\times 1$. Photographs by R. P. Marshall.

on clone OP-55. However, variations in susceptibility were indicated by the conspicuous blackening of the bark surrounding the wounds on clones OP-2, 46, 47, and 52, in contrast to a very slight discoloration on clones OP-4, 11, 12, and 41. The wound on clone OP-55 had not healed and the stem was

slightly depressed, but no bark discoloration was evident and the tissue cultures remained sterile.

The leaf spots resulting from the inoculations corresponded with those described and illustrated by Bier (1), and mature pycnidia with spores extruding in pinkish cirri were present about three weeks after inoculation.

Table 1 gives a summary of the results from the three series of inoculations with the isolate from the *Attea* specimens. In all controls the wounds healed rapidly and normally (Fig. 1, D).

TABLE 1.—*Results of inoculations of hybrid poplar clones with Septoria musiva*

Clone	Part inoculated					
	Stem wound		Leaves		Petiole	
	Inoc. ^a	Inf. ^a	Inoc.	Inf.	Inoc.	Inf.
	No.	No.	No.	No.	No.	No.
OP-1	4	1	2	1	2	0
OP-2	4	4	2	1 ^b	2	0
OP-4	4	1	2	2	2	1
OP-11	4	1	2	1	2	0
OP-12	4	1	2	0 ^b	2	0
OP-41	4	1	2	0 ^b	2	0
OP-46	4	3	2	1	2	0
OP-47	4	3	2	2	2	1
OP-52	4	3	2	0	2	0
OP-55	4	0	2	0	2	0

^a Inoc. = inoculations. Inf. = infections.

^b One leaf inoculation of clone OP-2 and OP-12, and both leaf inoculations on clone OP-41 were pruned by mistake before the results were observed.

DISCUSSION

The results from these few inoculations are not considered to be conclusive evidence of variations in susceptibility to stem infection among the clones tested, but they seem to indicate a high degree of susceptibility in clones OP-2, 46, 47, and 52. Of these, the three latter clones were derived from hybrids with *Populus maximowiczii* as one of the parents, and *P. berolinensis* as the other parent in two cases. The susceptibility of *P. maximowiczii* has not been tested, but *P. berolinensis* proved to be susceptible in the inoculations made by Bier (1). The high susceptibility of clone OP-2, with a parentage of *P. nigra* × *P. laurifolia*, indicates the desirability of testing these two parent species. However, clones with the same parentage may vary in susceptibility, as shown by the variation in inoculation results between clone OP-2 and clones OP-1 and 4. Bier also found that the Northwest poplar, a native hybrid, showed a high percentage of infection, equal to that of the exotic species *P. rasumowskyana*, while the native species from which the Northwest poplar was derived were resistant. He pointed out that *Septoria musiva* is an example of a native fungus of minor importance as the cause of a leaf spot on native species, which may act as a virulent parasite on new hosts, such as hybrids and exotic species.

The actual distribution of *Septoria musiva* in cankers on the various exotic species of poplar in the United States is not known. Apparently there have been no reports in literature of a similar canker disease other than those already mentioned from North Dakota. However, the prevalence of secondary fungi such as *Cytospora*, *Fusarium*, and *Phomopsis*, on cankers in the plantations and following inoculations, and the difficulty of isolating *S. musiva* from cankers thus infected, suggest the possibility that the disease may have been overlooked. In Canada (4) a perennial canker of unknown origin was described on Russian poplars, from which a species of *Cytospora* was isolated. The fungus was found particularly in callus tissue surrounding wounds and was considered to be responsible for the continuance of the disease. Moreover, Bier (1) stated that *Cytospora* was frequently isolated from the margins of older cankers that therefore might be the result of combined infection by *S. musiva* and the *Cytospora*. Pycnidia and spores of *S. musiva* are apparently produced during a very short period, even on the most susceptible trees. Infection by the fungus retards callus formation and, on the more resistant trees, creates conditions particularly favorable for infection by rapid-growing secondary fungi. This is evident from the fact that all of the wounds made in the controls of the inoculation series healed rapidly, with no infection by any fungus. It is possible, therefore, that on slightly susceptible poplars the growth of *S. musiva* might be retarded or even prevented by species of *Cytospora* or similar fungi.

Dothichiza populea, the cause of a disease of poplars, common in this country and Europe, was not found in any of the cankers examined from the New York plantations. However, clones of 10 of the hybrid poplars developed by Schreiner and Stout (11) were tested by Van Vloten (13) for susceptibility to *D. populea*. His results indicate that the clones most susceptible to that fungus were those of parentage similar to OP 2, 46, 47, and 52, which also proved highly susceptible to *Septoria musiva*.

SUMMARY

A canker disease of hybrid poplar clones in two plantings in New York State and in a planting made by the Tennessee Valley Authority at Norris, Tennessee, is caused by *Septoria musiva*. The fungus is indigenous in North America, occurring on living leaves of various species of native and exotic poplars in the United States and Canada. The canker disease has previously been reported only on exotic and hybrid poplars in Canada and Argentina.

Infection takes place through uninjured leaves and petioles, or through twig wounds. Cankers are formed on twigs of the current season's growth and pycnidia and spores appear soon after infection. The growth of the fungus from the twigs into the main stem results in the formation of cankers that, in highly susceptible trees, eventually girdle the stems. On less susceptible trees, the cankers may become infected by secondary fungi, such as *Cytospora*, which grow more rapidly than the *Septoria* and tend to mask the presence of the causal organism.

The relative susceptibility of 10 clones of hybrid poplars that have proved particularly adaptable for reforestation was tested by inoculations in the greenhouse and outdoors. Infection occurred in uninjured leaves and petioles, and in stem wounds. The results indicated a high degree of susceptibility in one clone with a parentage of *Populus nigra* \times *P. laurifolia*, two of *P. maximowiczii* \times *P. berolinensis*, and one of *P. maximowiczii* \times *P. nigra* var. *platicrensis*.

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THE ANTIGENICITY OF SOUTHERN BEAN MOSAIC VIRUS

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There is now abundant evidence that certain plant viruses are capable of acting as antigens and that their serological reactions are useful not only in ascertaining their relationships but also in providing a rapid means for their identification and determination of their concentration (1).

Recently, when southern bean mosaic virus (*Marmor laesiofaciens* Zaunmeyer and Harter (4)) was isolated in pure form (2), it seemed desirable to test its antigenicity and to determine its serological relationship, if any, to certain other plant viruses. This paper presents the results of studies carried out on the precipitin reaction of the virus with these objectives in mind.

Unless otherwise specified the procedure in the experiments was as follows: Two-tenths cc. of the appropriate dilution of antiserum was mixed with 0.2 cc. of a chosen dilution of antigen in a 50 × 6 mm. test tube. All the mixtures in an experiment were examined immediately after all the tubes had been set up. They were incubated at 37° C. and were reexamined after 1 hour and again after 5 hours at this temperature. Dilutions were made in 0.85 per cent NaCl solution. The concentrations given for antigen and antiserum are those in the reacting mixture.

In a preliminary experiment mixtures were prepared containing as one component about 0.4 mg. cc. of purified southern bean mosaic virus and as the second component antiserum at 1:15 for one or another of the following viruses: Potato ringspot (*Marmor dubium* H. var. *annulus* H.), potato veinbanding (*M. cucumeris* H. var. *upsilon* H.), tobacco mosaic (*M. tabaci* H. var. *vulgare* H.), tobacco etch (*M. erodens* H. var. *vulgare* H.), tomato bushy stunt (*M. dodcahedron* H.), and tobacco necrosis (*M. lethale* H.). These antisera were the only ones available at the time. Readings were made after keeping the tubes at room temperature for 1 hour and 4 hours and then after keeping them overnight at 7° C. The southern bean mosaic virus reacted with none of the antisera although each antiserum reacted strongly in control mixtures containing its homologous virus in plant juice cleared by low speed centrifugation. The experiment indicated that southern bean mosaic virus was not serologically related to any of these viruses.

Because southern bean mosaic virus resembled bushy stunt virus and tobacco necrosis virus in some of its properties a purified preparation of southern bean mosaic virus was then tested at concentrations of 0.5, 0.25, 0.125, 0.063, 0.031, 0.016, 0.008, 0.004, 0.002, and 0.001 mg. cc. against 1:18 dilutions of tomato bushy stunt and tobacco necrosis antisera and a 1:18 dilution of normal rabbit serum. There was no precipitin reaction at any dilution of the virus, again suggesting that southern bean mosaic virus was serologically distinct from tomato bushy stunt and tobacco necrosis viruses.

The next step was to prepare an antiserum to southern bean mosaic virus. The virus preparation used as antigen had been partially purified and concentrated by precipitating it with $(\text{NH}_4)_2\text{SO}_4$, removing the precipitate with low-speed centrifugation, and redissolving it in water. It was then further concentrated and purified by centrifuging it twice at 24,000 r.p.m. for 2½ hours to sediment the virus, taking it up in water after each centrifugation and centrifuging at 3000 r.p.m. for a few minutes to remove aggregated colloidal material. It was finally dialyzed against distilled water. The concentrated material contained 84 mg. cc. of virus and had an amber color at this concentration. It was found to be essentially homogeneous when tested in an analytical centrifuge, a Tiselius electrophoresis apparatus, and a diffusion apparatus.¹ Examination under an electron microscope showed it to consist of spherical particles of uniform size and to be essentially free of extraneous particles of other shapes or sizes (3).

TABLE 1.—*Titration of antisera to southern bean mosaic virus*

Serum	Serum dilution	Time (minutes)	Antigen concentration (mg./cc. of virus)	
			0.1	0.01
Antiserum from rabbit No. 1	1:10	15	+	—
		135	+++	±
		1440	+++	+
	1:100	15	+	—
		135	++	—
		1440	+++	++
Antiserum from rabbit No. 2	1:10	15	+	—
		135	+++	±
		1440	+++	++
	1:100	15	+	—
		135	++	—
		1440	+++	++

As a preliminary, 5 cc. of blood were withdrawn from the ear vein of each of the two rabbits to be used later in preparing the antisera. This blood furnished the normal sera which were to be used later in comparison with the immune sera. After waiting a week, each of the two rabbits was injected intraperitoneally with 2 mg. of virus dissolved in 4 cc. of 0.85 NaCl solution. After a ten-day interval each of the rabbits was bled, and the serum thus obtained was held at -10°C . until needed.

A purified preparation of southern bean mosaic virus was tested against the normal serum and the antiserum from each of the two rabbits. The mixtures were kept at 37°C . for 5 hours, after which they were kept at 7°C . for the next 19 hours. The reactions with the antisera are shown in table 1. Since there was no reaction of virus with either normal serum when tested at the same dilutions used for the antisera, nor any reaction when antisera were diluted 1:1000 or antigen diluted to 0.001 or 0.0001

¹ Data to be published.

mg./cc. of virus, these negative results are not included in the table. The data show that southern bean mosaic virus is antigenic and suggest that the serological reaction can be useful in testing for its relationships.

The next step was to test the specificity of the southern bean mosaic virus antiserum. The two viruses that most closely resemble southern bean mosaic virus in properties, tobacco necrosis and tomato bushy stunt viruses, were used in the tests. Purified preparations of these viruses were prepared by differential centrifugation and the final products were analyzed for protein content. A series of dilutions of each of these purified preparations and of a purified preparation of southern bean mosaic virus were tested against normal serum and against antiserum to each of the three. The dilution of antiserum in each mixture was 1:20. The data obtained are in table 2.

After adding the solutions of antigen and antiserum to all of the tubes and before mixing them, they were examined. Some tubes showed a definite cloudy disc where antiserum and antigen came in contact. Such a reaction is indicated in the table by a “:” sign in the space allotted to the 15-minute reading. The final data show that southern bean mosaic virus reacted with homologous antiserum, prepared by means of a single intraperitoneal injection of 2 mg. of virus, at a concentration as low as 0.01 mg./cc. but failed to react with the heterologous antisera at any concentration tested. Tobacco necrosis virus reacted only with its homologous antiserum and at a concentration as low as 0.05 mg./cc. Similarly, tomato bushy stunt virus reacted only with the homologous antiserum and at a concentration as low as 0.02 mg./cc. A second test set up in essentially the same manner gave practically identical results. These results indicate that each of the viruses is serologically distinct.

The data thus far presented show definitely that southern bean mosaic virus is antigenic and that its precipitin reaction is specific, so far as tested. These data were obtained entirely from tests made with purified preparations of virus. It seemed desirable to determine whether the juice from diseased bean plants would give a specific serological reaction when mixed with antiserum to southern bean mosaic virus. Juices expressed from ground, unfrozen, healthy and diseased Bountiful bean (*Phaseolus vulgaris* L.) plants were centrifuged for a few minutes at 3000 r.p.m. The juices were then tested at a series of dilutions in mixtures containing normal serum or antiserum to southern bean mosaic virus at a dilution of 1:20. After 1 hour at 37° C. there were specific precipitin reactions in the tubes containing homologous antiserum and diseased juice at dilutions 1:2, 1:4, 1:8, 1:16, and 1:32. No reaction occurred in control tubes containing juice from healthy plants and the antiserum or in those containing diseased juice and normal serum. After 5 hours at 37° C. the diseased juice up to a dilution of 1:128 exhibited a reaction with antiserum but by this time artifact reactions were developing in all tubes containing juice, whether diseased or healthy, at dilutions up to 1:8. A second test gave essentially the same results. These experiments show clearly that the antiserum reacts specifi-

TABLE 2.—*Specificity of antiserum to southern bean mosaic virus*

Serum ^a	Time (minutes)	Antigen concentration (mg./cc. of virus)				
		0.1	0.05	0.025	0.0125	0.0063 ^b
Antigen = southern bean mosaic virus						
Normal	15	—	—	—	—	—
	60	—	—	—	—	—
	300	—	—	—	—	—
Anti-southern bean mosaic virus	15	+	+	+	+	—
	60	++	+	+	+	—
	300	+++	+++	+	+	±
Anti-tobacco necrosis virus	15	—	—	—	—	—
	60	—	—	—	—	—
	300	—	—	—	—	—
Anti-tomato bushy stunt virus	15	—	—	—	—	—
	60	—	—	—	—	—
	300	—	—	—	—	—
Antigen = tobacco necrosis virus						
Normal	15	—	—	—	—	—
	60	—	—	—	—	—
	300	—	—	—	—	—
Anti-southern bean mosaic virus	15	—	—	—	—	—
	60	—	—	—	—	—
	300	—	—	—	—	—
Anti-tobacco necrosis virus	15	+	+	—	—	—
	60	+	+	—	—	—
	300	++	+	+	—	—
Anti-tomato bushy stunt virus	15	—	—	—	—	—
	60	—	—	—	—	—
	300	—	—	—	—	—
Antigen = tomato bushy stunt virus						
Normal	15	—	—	—	—	—
	60	—	—	—	—	—
	300	—	—	—	—	—
Anti-southern bean mosaic virus	15	—	—	—	—	—
	60	—	—	—	—	—
	300	—	—	—	—	—
Anti-tobacco necrosis virus	15	—	—	—	—	—
	60	—	—	—	—	—
	300	—	—	—	—	—
Anti-tomato bushy stunt virus	15	+	+	+	—	—
	60	++	+	+	—	—
	300	+++	+++	+	±	—

^a The serum in each mixture was diluted 1:20.

^b Negative results were obtained with virus concentrations of 0.0031 and 0.0016 mg./cc. and accordingly mixtures with these concentrations were omitted from the table.

cally with the juice from diseased plants but not with that from healthy plants. The experiments also indicate that the original purified virus used in preparing the antiserum was essentially free of protein constituents of the normal plants.

The results here reported are of interest not only because they show that southern bean mosaic virus is antigenic and that the precipitin reaction can be of use in testing for its virus relationships but also because they bring additional evidence for the belief that southern bean mosaic virus is an independent and distinct virus species. It has been shown elsewhere (3) that this virus can be distinguished from most other plant viruses by its high degree of thermostability and that in plant protection tests it is immunologically distinct from tobacco mosaic and tobacco necrosis viruses, both of which it resembles in thermostability. Hitherto, it was differentiated from tomato bushy stunt virus mainly on the striking differences in host range but partly also because of differences in physical and chemical properties. Southern bean mosaic virus has now been demonstrated to be distinguishable serologically from tomato bushy stunt virus.

SUMMARY

It is shown that southern bean mosaic virus is antigenic and that its precipitin reaction is useful in distinguishing it from other viruses. Southern bean mosaic virus is serologically distinct from tobacco necrosis virus and tomato bushy stunt virus, which it resembles in some of its physical properties. It is also serologically distinct from potato ringspot, potato veinbanding, tobacco mosaic, and tobacco etch viruses. Its antiserum, prepared with purified virus, reacts specifically with juice from Bountiful bean plants infected with the virus. The data bring additional evidence for the view that southern bean mosaic virus is an independent and distinct virus species.

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PRINCETON, NEW JERSEY.

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PHYTOPATHOLOGICAL NOTES

*Arasan for Control of Fungi in Germinating Corn Seed.*¹—That Arasan inhibits the development of *Diplodia zeae* (Schw.) Lév. has been shown by Hoppe² in a study of infected corn seeds placed on agar surfaces. He found that Arasan was equal to Barbak C and Semesan Jr. and superior to Spergon in retarding the growth of *D. zeae*. There is also evidence³ that in laboratory germination tests *D. zeae* is more effectively controlled with Arasan than with dusts containing ethyl mercury phosphate, ethyl mercury p-toluene sulfonanalide, or 2-3 dichloro, 1-4 naphthoquinone. Arasan applied to samples of low-germinating corn at the rate of 0.15 per cent by weight, however, did not control *Rhizopus nigricans* Ehr. as successfully as it did *D. zeae*. In contrast to these results, heavier dosages of Arasan prevented *R. nigricans* and other molds from growing on germinating seeds of cucumber, muskmelon, and squash.⁴

Apparently a proper dosage of Arasan should control both pathogenic and saprophytic fungi on germinating corn seed. To obtain information on this subject six selected dealers' lots of corn seed infested with *Diplodia zeae*, *Fusaria*, and *Rhizopus nigricans* and four lots infested with *R. nigricans* were treated with certain mixtures of Arasan and flour.

The mixtures (Table 1) contained 100, 67, 50, and 33 per cent Arasan and 0, 33, 50, and 67 per cent, respectively, of wheat flour. A known weight of seeds was agitated with one of the dusts for three minutes. The material that did not adhere was removed by shaking the treated seeds in a sieve. Arasan adhered at the rates of 2.3, 1.3, 0.9, and 0.6 ounces per bushel of seed for the 100, 67, 50, and 33 per cent Arasan mixtures, respectively.

The treated seeds and the controls were counted into replicates of 100 and were either germinated at once or stored in a dry laboratory for one, two, or three months before being germinated. All seeds were germinated on moistened paper towels at alternating temperatures of 20° and 30° C. Records were taken during the sixth day of germination.

Neither Arasan alone nor any of the Arasan-flour mixtures retarded the germination of any seed lot. Approximately 90 per cent of the live seeds had formed normal seedlings after only three days on the moistened towels regardless of the dosage of Arasan or the length of the storage period. In germination the control excelled any one treatment in only 29 of the 140 separate tests. The average results from all of the tests showed that the percentage germination of untreated seed was not higher than that of seed

¹ Journal Paper No. 613 of the New York State Agricultural Experiment Station, Geneva, New York.

² Hoppe, P. E. Comparison of certain mercury and non-metallic dusts for corn seed treatment. *Phytopath.* **33**: 602-606. 1943.

³ Crosier, Willard. Chemical control of molds, bacteria and fungus pathogens on cucurbit, pea and sweet corn seeds. *Proc. Assn. Off. Seed Analysts* **1943**: 38-41. 1945.

⁴ Crosier, Willard. Chemical seed protectants used as disinfectants during germination of cucurbit seeds. *News Letter Assn. Off. Seed Analysts* **18**(2): 3-6. 1944.

treated with any Arasan mixture or with Arasan alone. This was true for all ten lots of corn.

Rhizopus nigricans is readily controlled in viable, fairly clean seed (Table 1). In heavily infested seed containing 8 to 20 per cent dead kernels, however, *R. nigricans* appeared to be beneath the pericarp of some seed and hence not exposed to surface sterilization. The heavier concentrations of Arasan (100 and 67 per cent) excelled the lighter concentrations (50 and 33 per cent) in controlling fungi in this type of corn seed.

While neither *Diplodia zeae* nor *Fusaria* were present in a high percentage of seeds, it is apparent (Table 1) that Arasan retarded the develop-

TABLE 1.—Laboratory germination, seedling weight, and fungus contaminants of corn seed treated with certain mixtures of Arasan and wheat flour

Arasan flour mixture	Normal seedlings		Seeds or seedlings contaminated with								Green weight of seedlings in entire test	
			<i>Rhizopus nigricans</i>		<i>Diplodia zeae</i>		<i>Fusaria</i>					
	Pct.	Rank ^a	Pct.	Rank	Pct.	Rank	Pct.	Rank	Pct.	Rank	Grams	Rank
Averages for six seed lots infested with <i>Diplodia</i> , <i>Fusaria</i> , and <i>Rhizopus</i> ^b												
100-0	90	66	4	75	Tr.	81	1	59			78	54
67-33	90	57	5	76	Tr.	67	1	61			79	64
50-50	90	64	11	62	Tr.	59	1	67			80	66
33-67	88	46	27	32	1	35	1	53			79	56
0-100	86	17	48	5	5	8	4	10			72	10
Averages for four seed lots infested with <i>Rhizopus</i> ^c												
100-0	97	27	Tr.	26							78	21
67-33	96	15	0	29							78	23
50-50	97	24	Tr.	27							78	24
33-67	97	22	1	18							79	27
0-100	96	12	21	0							72	5

^a Rank is based upon total number of items in which an Arasan-flour mixture (or control) proved superior to any other mixture (or control). Normally each mixture (and control) would be ranked from 0 to 4 in ascending order of superiority.

^b Twenty-six replicated tests; 100 possibilities for superiority.

^c Twelve replicated tests; 40 possibilities for superiority.

ment of these fungi. When these fungi did develop on Arasan-treated seed the colonies were markedly smaller than those developing from untreated seed.

Chemical injury either was not apparent or was very slight. In a bulk lot of surface-cracked seeds treated with Arasan alone 2 per cent of seedlings were slightly injured. No injury was noticed in germinating seeds of other corn lots.

The average green weights of seedlings in the 100-seed tests prove that Arasan either did not cause any chemical injury or the increases in weight attributable to control of fungi were greater than the decreases effected by chemical injury. The data in table 1 show, furthermore, that the treatments not only did not depress, but actually resulted in increased green weights of the seedlings. The data do not indicate, however, whether the increases in green weight are due to control of the fungi, the effect of the toxicant

content of Arasan, or the effect of the diluent present in Arasan and the Arasan-flour mixtures upon the seedlings.

In two other bulk lots of seeds carrying only traces of fungi the average green weights of seedlings from ten replicated tests of each treatment were: 100 per cent Arasan—76 grams, 50 per cent Arasan—76 grams, 33 per cent Arasan—77 grams, and no treatment—72 grams. It does not appear probable that the differences in weights between the treated and untreated seeds were caused by control of fungi alone.—WILLARD CROSIER, Assistant Professor, Division of Seed Investigations, Cornell University, Geneva, New York, and STEWART PATRICK, Research Associate, Division of Seed Investigations, Cornell University, Geneva, New York.

*Internal Brown Spot, a Boron Deficiency Disease of Sweet Potato.*¹—In exploring certain possible causes of internal cork,² a newly described disease of sweet potato (*Ipomoea batatas* (L.) Lam.), the likelihood that boron deficiency, or some other nutritional agency, might be involved was investigated in 1944. In one field experiment of complex design sweet potatoes of the Porto Rico variety were grown on replicated field plots, located on both limed and unlimed soils at three different levels of potassium fertilization and supplied with borax at rates of 0, 5, 10, 20 and 30 pounds per acre. Although there was no correlation between the incidence of internal cork and the response of the sweet-potato plants to these nutritional treatments, this experiment, nevertheless, afforded an opportunity for studying certain physiogenic abnormalities of plants grown on no-borax plots. The induced disease is herein referred to as "internal brown spot" because it is characterized primarily by the occurrence of brown necrotic areas in the flesh of the roots. Inasmuch as the trouble has not been observed on plants supplied with borax, it has been attributed to boron deficiency. This report deals only with field symptoms of the disease and is offered preliminary to a more comprehensive paper to be published upon the completion of work now in progress. A brief description of some of the symptoms noted herewith has already been published.³

The symptoms on the vines and leaves were not detected until the latter part of August, about two months after planting, when some of the plants on no-borax plots showed a restriction of terminal growth and shortening of the internodes. As the season advanced these symptoms developed in varying degree on nearly all of the plants on all no-borax plots. As the disease continued to develop, the petioles became curled and the terminals became stunted and distorted. In October many of the terminals died and vine growth ceased. The older leaves turned yellow and shed from the vines, exposing the crowns of the plants. Partial collapse of vines near the crown,

¹ Technical contribution number 128 of the South Carolina Agricultural Experiment Station.

² Nusbaum, C. J. Internal cork, a new disease of sweet potato of unidentified cause. *Phytopath.* 36: 18-23. 1946.

³ Nusbaum, C. J. Studies of the use of boron with sweetpotatoes. S. C. 57th Ann. Rept., 144-149. 1945.

probably due to sunburn, was not uncommon. A comparison of vines on boron-deficient and healthy plants is shown in figure 1. These photographs were taken in mid-October just before harvest. Both plants represented



FIG. 1. Comparison of vine growth on boron-deficient and healthy plants, both of which were grown in a high-calcium, high-potassium block. A. Plant grown upon no-borax plot. Note the gnarled growth characterized by shortened internodes, curled petioles, defoliation of basal portion of the vine, and distorted terminal. B. Plant grown upon a plot supplied with borax at the rate of 10 pounds per acre. Note the long unrestricted vine growth, the more or less regularly spaced nodes, and the straight petioles.

were in the high-lime, high-potassium block. The deficient plant (A) shows the typical gnarled growth characterized by short, irregular internodes, curled petioles, dead terminal and abscised lower leaves. The healthy plant

(B), which received borax at the rate of 10 pounds per acre, shows long, unrestricted vine growth with more or less regularly spaced nodes and straight petioles. At harvest the terminals of such vines were still elongating.

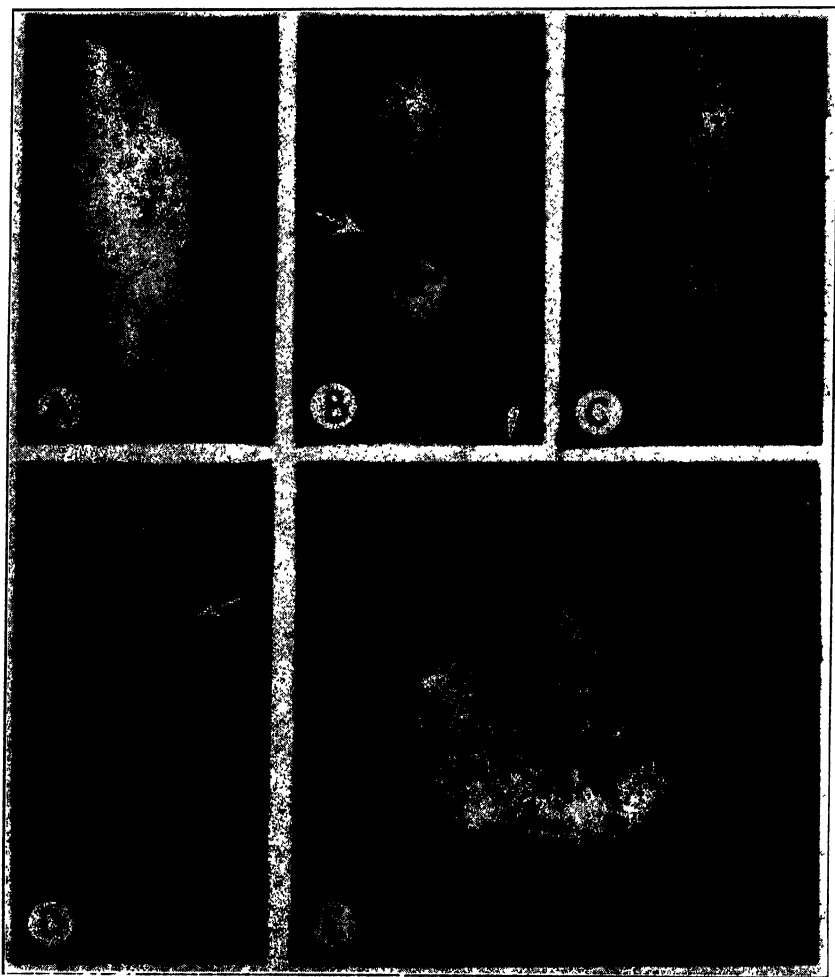


FIG. 2. Sweet-potato roots produced in a low-calcium, high-potassium block. The healthy root (A) was produced on a plot which received borax at the rate of 5 pounds per acre. Note the symmetrical shape and smooth appearance. The external appearance of typical diseased roots is shown in a dumbbell-shaped root (B) with an arrow pointing to an exudate-covered canker, a lop-sided root (C), and a spindling root (D) with a long surface canker, without exudate, indicated by an arrow. E. Cross section of a diseased root from a no-borax plot. Note the uneven outline of the root and the appearance of the indistinct spotting, especially in the cambial zone near the periphery.

At digging time the roots from no-borax plots showed varying degrees of both external and internal degeneration. In outward appearance they were misshapen and the skin was rough and leathery in texture somewhat resembling buckskin. Many of the roots appeared not to have filled out at certain

points, resulting in dumbbell-shape, lopsided or spindle-shape malformations, such as those shown in figure 2, B, C and D. A smooth, well-formed root taken from a plot which received 5 pounds of borax per acre is shown in figure 2, A, for comparison. Severely affected roots usually showed surface cankers which were sometimes covered with a hardened and blackened exudate (Fig. 2, B), the origin or nature of which has not been ascertained. The fleshy root shown in figure 2, D, however, shows an elongated canker, indicated by an arrow, without this exudate. Roots with the external symptoms described usually, but not always, had a characteristic internal breakdown, as shown in figure 2, E. It always was found, however, in severely misshapen roots or in those with surface cankers.

The internal necrotic areas were variable in size and brown with indistinct margins. They occurred indiscriminately throughout the flesh but seemed to be most prevalent in the cambial zone near the periphery of the root. The origin of the spots or the tissues involved has not been determined. The flesh of roots with internal brown spot was generally of poorer color and of softer texture than that of healthy roots.

The possible nutritional rôle of boron in the sweet potato was first reported by Willis,⁴ who pointed out that small applications of borax often prevented cracking of the fleshy roots and improved the flavor and texture of the flesh. He also suggested that darkening of the sweet potatoes, hitherto attributed to damage from chilling at temperatures above freezing, could be eliminated or reduced by the use of borax in the fertilizer. In the studies here reported cracked roots occurred in small amounts in nearly all plots regardless of rate of borax applied. Furthermore, the internal brown spot symptom here described developed in sweet potatoes which had not been exposed to temperatures below 50° F., either in the field or in storage, and was distinctly different from the discoloration and subsequent breakdown of the flesh commonly attributed to chilling injury. Reave *et al.*⁵ state briefly that sweet-potato plants grown in the greenhouse without boron produced roots having "dark streaked centers" but clear-cut boron deficiency symptoms were not observed in the field.—C. J. NUSBAUM, Edisto Experiment Station, Branch of Clemson College, Blackville, S. C.

A Rapid Method for Isolating Single Ascospores from Apothecia.—This note deals with a subject with which the writer has not been in contact for many years but it is impelled by the recent death of Professor H. H. Whetzel. While serving as his assistant in 1917 the writer discovered a rapid method for isolating single spores from the apothecia of various species of *Sclerotinia* and related genera. The procedure for getting single-spore cultures as taught the writer by Professor Whetzel consisted in suspending an apothecium within and from the top of a large bell-jar so that the hymenial disc

⁴ Willis, L. G. Apply borax to improve quality of sweet potatoes. N. C. Agr. Exp. Sta. Special Circ. 1. 1943.

⁵ Reave, Eldrow, A. L. Prince, and F. E. Bear. The boron needs of New Jersey soils. N. J. Agr. Exp. Sta. Bul. 709. 1944.

faced downward. An open Petri dish containing nutrient agar was then placed within and at the base of the bell-jar to catch the spores that floated down. The apparatus was left thus overnight. Later, the Petri dish was examined microscopically and young cultures from individual spores were transferred to tubes. This method got results but it was laborious and favorable to contaminations. Not infrequently only very few ascospores were shed. In an effort to improve the method, the writer tried immersing the lower two-thirds of an apothecium in 95 per cent alcohol. Immediately a cloud of ascospores was ejected. Taking advantage of this action, single ascospores were isolated simply by placing three Petri dishes containing nutrient agar near a vial with enough 95 per cent alcohol to cover the lower two-thirds of the apothecium when this was dropped into the vial. In order not to interfere with spore dispersal, vials were selected that were not much taller than the fruiting bodies and in order to keep the apothecia in an upright position to avoid getting alcohol on the hymenium, vials were selected that were only slightly larger in diameter than the apothecia. Immediately following immersion and the ejection of a cloud of ascospores, a Petri dish was momentarily opened in the cloud. This was quickly repeated with the second and third Petri dishes. Usually the last caught only a few scattered spores in perfect placement for isolation. Due to their short exposures, the plates usually were wholly free from contaminations.

Among the many apothecia collected by Professor Whetzel were small ones, only a few millimeters in diameter, from which it was very difficult to obtain spores by the bell-jar method. In applying the principles of the new technique, forceps were used to hold these apothecia face downward over nutrient agar in an open Petri dish. A needle that had been dipped in alcohol then was lightly rubbed against the outer layer of the fruiting body. This brought about an immediate ejection of ascospores.

The advantages of the new method are rapidity, excellent spore dispersal, freedom from contamination, and applicability to the smallest apothecia. Possibly the method may be applicable to some other fungus fruiting bodies.—V. F. TAPKE, Plant Industry Station, Beltsville, Maryland.

*Induced Baldhead in Soybean.*¹—For five years the Iowa State College Seed Laboratory has been investigating the possibility of indexing commercial lots of seed for their reaction to low temperature in *Pythium*-infested soil at different moisture levels in terms of their response when planted in the field under conditions unfavorable to germination. In addition, a study has been made of the relative ability of seed protectants to prevent seed decay and seedling blight when seeds are germinated in *Pythium*-infested soil. In April, 1944, treated and nontreated seeds of Bansei soybean were planted in a mixture of *Pythium*-infested soil and sand with moisture content of about 15 per cent, according to the procedure described by Rice² and

¹Journal Paper No. J-1318 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 427.

²Rice, N. W. The development and evaluation of seed laboratory methods for determining the presence and significance of certain soil-borne and seed-borne organisms. Unpublished doctor's thesis, Iowa State College Library. 1944.

TABLE 1.—Percentages of normal and "baldhead" seedlings from treated and nontreated soybean seed planted in *Pythium*-infested soil, Iowa, 1945

Treatment	7 days cold soil				10 days cold soil			
	Kanro		Lincoln		Kanro		Lincoln	
	N ^a	B ^a	N	B	N	B	N	B
Nontreated	49	8.2	43.8	39.2	52.8	21.8	39.5	37.2
Sem. Jr.	70	7.2	72.8	19.5				
Spergon	83	1.2	95.0	2.2				
Arasan	85	0.2	98.2	0.5	89.8	0.5	95.8	0.75

^a N = Normal seedlings, B = Baldheads.

Porter.³ The soil used was naturally infested with *Pythium graminicola* Subr. and *P. de Baryanum* Hesse. The flats containing the seeds were placed in a room with a constant temperature of 10° C. for 7 days, then transferred to an adjoining room at 26° to 28° C. At the end of 5 days in the latter room the seedlings were examined and classified as emerged, normal, and abnormal. Fungicides used were Arasan, Spergon, and Fermate.

Among the seedlings classed as abnormal were a number, produced by nontreated seed, that resembled "baldheads."^{4, 5} Close examination revealed



FIG. 1. Seedlings of Lincoln soybean grown in *Pythium*-infested soil at 10° C. for seven days followed by exposure at 26–28° C. for five days. Left, treated with Arasan. Right, not treated.

³ Porter, R. H. Testing the quality of seeds for farm and garden. Iowa Agr. Exp. Sta. Res. Bul. 334. 1944.

⁴ Borthwick, H. A. Thresher injury in baby Lima beans. Jour. Agr. Res. [U.S.] 44: 503–510. 1932.

⁵ Harter, L. L. Thresher injury a cause of baldhead in beans. Jour. Agr. Res. [U.S.] 40: 371–384. 1930.

that the plumule had failed to develop because necrosis of the primary leaves had occurred early in the growth of the plumule. In many cases axillary buds had developed at the base of the decayed plumule in seedlings that were three or four inches high. Of 400 nontreated seeds planted, 18.2 per cent produced "baldheads." The percentage of baldheads from treated seed was 1.4 for Arasan, 0.65 for Spergon, and 4.4 for Fermate. This particular lot of seed when tested in sand at 26° to 28° C. gave 99 per cent normal seedlings with no baldheads.

In April, May, and June, 1945, seeds of Kanro and Lincoln soybeans from the 1944 crop were selected for test. Tests in sand at 26°–28° C. gave 95.5 per cent normal seedlings for Kanro and 98.0 per cent normal for Lincoln. No "baldheads" were produced. Treated and nontreated seed of these two lots was then planted in *Pythium*-infested soil and held for 7 or 10 days at 10° C., then transferred to a room at 26°–28° C. The counts of emerged seedlings gave results as shown in table 1.

In figure 1 are shown a few normal and "baldhead" seedlings of the variety Lincoln.

To determine the effect of low temperature alone seed of both varieties was planted in sterilized sand and the flats were held 10 days at 10° C. before transfer to the warm room. The results showed one per cent "baldhead" seedlings each from nontreated seed and Arasan-treated seed, respectively. The percentages of normal seedlings were 93 and 94 for treated and nontreated, respectively.

The data herein summarized indicate that soybean seed of high germinability when planted in *Pythium*-infested soil with a moisture content of 15 per cent and retained at 10° C. for 7 or 10 days may be expected to produce a high percentage of "baldhead" seedlings in which the plumule is either killed or partially decayed.—R. H. PORTER, Iowa State College, Ames, Iowa.

Relative Resistance and Susceptibility of U.S. 243 and U.S. 343 Lima Beans to Lima-Bean Mosaic.—During the summer of 1945 two promising strains of Lima beans were being increased for distribution under the designations U.S. 243 and U.S. 343. Trial plantings were also made at numerous experiment stations¹ and in a few home gardens. In June a diseased specimen of U.S. 343 apparently affected with mosaic was brought to the attention of the writers. An examination of the garden from which this plant came showed that two 20-foot rows, one of U.S. 243 and one of U.S. 343, had been planted end to end. Of the 49 plants in the row of U.S. 343, 33 had mosaic; while all of the 33 plants in the row of U.S. 243 appeared to be free of the disease. Inoculations to appropriate hosts indicated that the mosaic was caused by a virus similar to the one described by Harter.² Greenhouse inoculations (Table 1) substantiated the field observations. Inoculations

¹ Magruder, Roy, and R. E. Wester. A preview of two new bush lima beans. *Market Growers Jour.* 74: 23. 1945.

² Harter, L. I. Mosaic of lima beans (*Phaseolus lunatus macrocarpus*). *Jour. Agr. Res.* [U.S.] 56: 895-906. 1938.

TABLE 1.—*The relative susceptibility of U.S. 243 and U.S. 343 to lima bean mosaic*

Strain	Noninoculated		Inoculated	
	Total No. plants	No. diseased	Total No. plants	No. diseased
U.S. 243	33	0	87	0
U.S. 343	97	0	145	93

from U.S. 243 to tobacco showed that this Lima strain was resistant and not a symptomless carrier of the virus.



FIG. 1. U.S. 343, susceptible to Lima-bean mosaic.

The resistance of U.S. 243 and susceptibility of U.S. 343 (Fig. 1) is an interesting case since both these lines are selections from the same Fordhook \times Sieva cross. Harter demonstrated that Fordhook is resistant to the virus; while Sieva is susceptible. Apparently in developing the two Lima strains, a resistant and a susceptible line were isolated by chance. This situation is another example^{3, 4} of how, in selecting for desirable horticultural characters, genes for resistance may be lost unless particular attention is given to the pathological phases of the breeding program.

Mosaic in Lima beans has never been serious in the past, even in the case of susceptible varieties, and is therefore likely to be of only technical interest for some time.—DEAN E. PRYOR and ROBERT E. WESTER, Plant Industry Station, Beltsville, Md.

³ Pryor, Dean E. A unique case of powdery mildew on lettuce in the field. U. S. Dept. Agr., Plant Disease Reporter **25**: 74. 1941.

⁴ Whitaker, Thomas W., and Dean E. Pryor. Genes for resistance to powdery mildew in *Cucumis melo*. Proc. Amer. Soc. Hort. Sci. **41**: 270-272. 1942.

ANNOUNCEMENT

The Statler Hotel will be headquarters for the meetings of the American Phytopathological Society at St. Louis, Missouri, March 27-30, 1946.

Make reservations before March 17 by writing to the Housing Bureau of the American Association for the Advancement of Science, 900 Syndicate Trust Building, St. Louis 1, Missouri. All requests for reservations must specify: (1) first, second, and third choice of hotel, (2) approximate price of room desired, (3) date and hour of arrival and departure, (4) society affiliations, (5) names and addresses of persons who will occupy the reservations.

<i>Hotel</i>	<i>One Person</i>	<i>Two Persons</i>		<i>2-Room Suites Parlor and Bedroom</i>
		<i>Double Bed</i>	<i>Twin Beds</i>	
Statler	\$3.50-5.00	\$5.00-7.00	\$6.50-9.00	
American	\$2.00-3.00	\$3.50-4.00	\$5.00	
Claridge	\$3.00-4.00	\$4.00-6.50	\$5.00-6.50	\$10.00 & up
Coronado	\$3.00 & up	\$5.00 & up	\$6.00 & up	\$ 8.00-13.00
DeSoto	\$2.65-7.00	\$4.00-7.00	\$5.30-10.00	\$10.00
Gatesworth	\$3.50	\$5.50	\$6.00	
Jefferson	\$3.50-5.00	\$4.50-6.00	\$6.00-8.00	\$12.00-20.00
Lennox	\$3.00-5.50	\$5.50-6.00	\$5.50-6.50	\$10.00-11-50
Majestic	\$2.00-2.25	\$2.75-3.25	\$4.00	
Mark Twain	\$2.75-3.50	\$4.00- 5.00	\$4.50-5.50	
Mayfair	\$3.00-6.50	\$4.00-8.00	\$5.50-8.00	\$10.50 & up
Melbourne	\$3.20-4.20	\$5.30-6.80	\$5.30-7.30	
Roosevelt	\$3.00	\$3.50	\$4.50	

All reservations must be made through the Housing Bureau. A limited number of accommodations are available and it is suggested that rooms to be occupied by two or more persons be requested in your reservations.

MERTON BENWAY WAITE

1865-1945

JOHN W. ROBERTS

The death of Merton Benway Waite on June 5, 1945, marked the passing of another of those early workers who, by the brilliance of their discoveries, helped to create the Bureau of Plant Industry and to establish public confidence in the usefulness of plant pathology.

Born January 23, 1865, on a farm near Oregon, Illinois, in the botanically interesting Rock River country, he developed an interest in plants while still in high school, an interest that was paramount throughout the rest of his life.

When he entered the University of Illinois, from which he was graduated in 1887, his parents hoped he would take a civil engineering course, but his love for plants and the influence of Dr. T. J. Burrill, Professor of Botany, soon prevailed. After graduation he became an assistant to Burrill, but Galloway, in his hunt for promising young men for his Section of Vegetable Pathology, soon chose him and from then, November 1, 1888, until his retirement in 1935, Waite was a member of that organization and its successor, the Bureau of Plant Industry. From 1901, he was in charge of the Office, later Division, of Fruit Disease Investigations. He brought to the pathologists of the Department the technic of isolating and growing bacteria and fungi in pure culture on artificial media, which he had learned under Burrill, and taught it with characteristic enthusiasm not only to his colleagues in the Department but to many visiting investigators from colleges and universities as well. One of his greatest pleasures was in teaching others these methods and his own improvements.

Waite will always be known for his work on blight of pomaceous fruits. He first demonstrated the dissemination of the pathogen by insects, an epoch-making discovery in the field of disease control. As an outgrowth of this work with pear blight and primarily because of his keen powers of observation, he also discovered that most varieties of pear required cross-pollination with other varieties to set an adequate crop of fruit. His proof of this fact is a model of exact experimental work that soon convinced the skeptics. This discovery, soon extended to other fruits, has profoundly affected orchard planting throughout the world. Fifty years ago at the frantic request of grower organizations Waite organized and led a campaign of pear-blight eradication, using methods largely originated by himself, that saved the pear industry of the Pacific Coast States from probable destruction. The methods used and taught by him are used today with only minor changes. A few years later and with the exercise of great patience he convinced the hardheaded apple growers of the Shenandoah Valley that the destruction of cedar trees would prevent defoliation by cedar rust, thus laying the foundation for the present cedar-rust law. His knowledge of fruit



MERTON BENWAY WAITE
1865-1945

diseases was probably beyond that of any investigator of his day, but in his later years he published little. To his visitors and colleagues, however, he always gave bountifully of his knowledge.

Waite helped organize and was a member of the Federal Insecticide and Fungicide Board, which administered the Insecticide law of 1910, until 1924, when he relinquished membership in this Board to become a member of the Federal Horticultural Board, which established and enforced the Federal quarantine regulations until its functions were taken over by another agency. On both these boards his services were distinguished, not only by his knowledge of plants, plant diseases, and horticultural practices, but by his scientific attitude and fairness.

In 1919 he was given the honorary degree of Doctor of Agriculture by the University of Maryland. He was a charter member of The American Phytopathological Society, Fellow of the American Association for the Advancement of Science, and a member of the Washington Academy of Science and other scientific societies. He helped to found and was twice president of the Botanical Society of Washington, in which he took a great interest up to the very day he was stricken. He seldom missed a meeting and usually furnished the flowers for the annual dinner.

Possessed of a pleasant disposition and always smiling, Waite was popular with his associates and always willing to be helpful to them. His zest for living, which continued to the day of his final sickness, makes it hard for us to realize that he is gone. His restless energy gave an impression of indestructibility that made death seem impossible.

In 1944, at the age of 79, he revisited his former collecting grounds in Illinois with the enthusiasm of youth and returned to Washington with an interesting account of the botanical changes and the finding of certain rare plants just where he expected them to be.

Those of us who were his associates for many years will miss him, not only because of his scientific attainments but because of his enthusiasm, his kindness, and an optimistic view of life that lasted to the very end.

He is survived by his wife, Elizabeth Hurdle Waite, and two sons, Merton and Malden, both of whom are captains in the armed forces.

BOTANICAL WRITINGS OF M. B. WAITE

(Compiled by J. A. Stevenson and Edith K. Cash)

- Results from recent investigations in pear blight. *Bot. Gaz.* **16**: 259. 1891.
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THE LIFE HISTORY OF THE GOLDEN NEMATODE OF POTATOES, *HETERODERA ROSTOCHIENSIS* WOLLENWEBER, UNDER LONG ISLAND, NEW YORK, CONDITIONS¹

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INTRODUCTION

At the present time there are four nematodes known to cause damage to potatoes in the United States: the root-knot nematode (*Heterodera marioni* (Cornu) Goodey) and Scribner's meadow nematode (*Pratylenchus scribneri* Steiner in Sherbakoff & Stanley), both prevalent throughout the potato acreages of the country; the potato-rot nematode (*Ditylenchus destructor* Thorne), occurring in a single area of Idaho; and the golden nematode of potatoes (*Heterodera rostochiensis* Wollenweber), occurring in a single area of Long Island, N. Y. The first two are prevalent and commonly cause considerable loss in crop on Long Island. *H. rostochiensis* causes considerably more damage in the area of its occurrence but is of minor importance when national crop losses are considered. The potential damage which could be expected from it, were it general, is our primary consideration, for it causes major losses throughout the entire northern potato-growing regions of Europe and the British Isles. Leiper² states concerning this disease: "Once its nature and importance had been realized, reports of its occurrence multiplied so rapidly that, by the early post-war period, practically all the potato-growing districts of Britain were known to have foci of infection. Since then infection has progressively increased in intensity so that to-day much of the potato land is ceasing to be economically productive, and consequently its once high market value has markedly depreciated."

While the golden nematode of potatoes was not discovered in the United States until 1941, our observations on the rate of its multiplication and spread indicate that it was introduced into Long Island around 1930. By 1934 there were a few isolated spots showing poor potato growth and by 1938 these spots had multiplied and requests for soil analysis had been made by the growers. In 1941 the potato growth was poor throughout the field on which these spots had been observed, and it was at that time that the causative organism was identified. In 1942 a cooperative survey showed infestation in 14 fields totaling 434 acres, in 1943 as many as 23 fields totaling 840 acres, and in 1944, 26 fields totaling 918 acres. In the meantime the State

¹ Investigations on this potato pest are carried on by the Division of Nematology, Bureau of Plant Industry, Soils and Agricultural Engineering, and the Division of Domestic Plant Quarantines, Bureau of Entomology and Plant Quarantine, both of the Agricultural Research Administration, U. S. Department of Agriculture; the Agricultural Experiment Station at Cornell University; and the New York State Department of Agriculture.

² Leiper, R. T. The potato eelworm problem of to-day. Jour. Roy. Agr. Soc. England 100(3): 63-73. 1940

of New York has enacted certain regulations to prevent local and national dispersal of the pest.

During the summer of 1941 we participated in a survey of the potato growing areas of the northeastern United States, which was conducted co-operatively with the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. In this survey 1480 farms, totaling 48,156 acres, were inspected for the presence of *Heterodera rostochiensis*. Although the survey represented a general sampling of the potato lands of 19 States, not a single instance of golden nematode was observed. While our survey could embrace only the examination of representative samples and, while negative findings are never conclusive, it can be stated that the odds are 99:1 that less than 4 farms in 1,000 and less than 2 acres in 10,000 are infested in the areas surveyed. It would appear that *H. rostochiensis* is wholly or chiefly confined to a single area of Long Island at the present time. From the national standpoint this area of infestation is important as a source of new infestations and as a threat to the potato industry of the country.

LIFE HISTORY OF THE NEMATODE

Eggs and larvae of *Heterodera rostochiensis* overwinter in the body of the dead female which has been transformed into a brown, thick-walled, protective cyst. In spring the eggs hatch and larvae emigrate from the cysts to attack potato roots. This activity is at least partially dependent upon temperature, and will be discussed later. The invading larvae penetrate any part of the root system or tubers, taking up a position with the head near the vascular system. The posterior part of the body may completely or only partially enter the root. Figure 1 shows the various stages of nematode development. The first and second molts are passed through rapidly (probably within 5 to 7 days after invasion) and there is little change in gross form during this period, though we believe the sexes can be distinguished through the thickening of the rectum in the male. Contrary to the development in *H. marioni* as given by Christie and Cobb,³ no molt appears to take place in the egg. We believe two molts take place in the larviform or slender period, evidence being a difference in the form of the cast-off portion of the stylet (Fig. 1, D1 ♂ & C ♀) and the coarser striation of the first-stage cuticle.

During the third stage (approximately 10 days after invasion) the posterior part of the body enlarges in both sexes, and may at this time break through the root epidermis if the specimen is lodged in a small root or near a root surface. During the fourth stage (also about 10 days) the posterior part of the body of the female continues to enlarge, very rapidly becoming spheroid, and many more specimens break through the root surface. The fourth stage male elongates, reassuming the vermiform shape, coiled within the third stage cuticle. During the latter part of the fourth stage the thick

³ Christie, J. R., and Grace Sherman Cobb. Notes on the life history of the root-knot nematode, *Heterodera marioni*. Proc. Helminthol. Soc. Washington 8: 23-26. 1941.

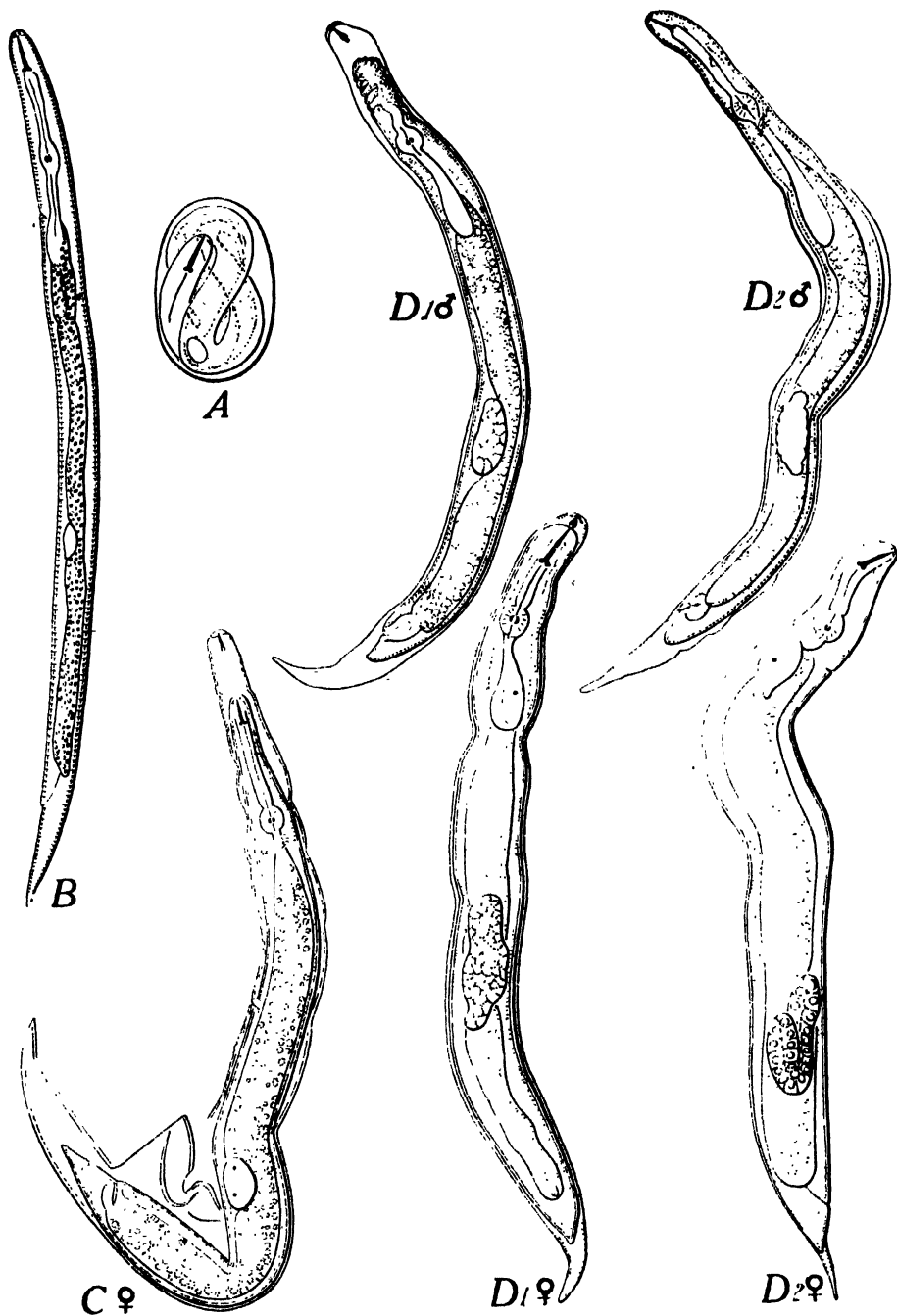


FIG. 1. Stages in the life history of *Heterodera rostochiensis*. Lettering and numbering arranged to identify corresponding stages in both sexes. All about 300 μ .

A—Egg containing larva, sex undeterminable.

B—Larva broken out of egg (1st stage), sex undeterminable.

D1 ♂ — Larva (? male) in early phase of 2nd molt. C ♀ — Larva (? female) in 1st molt.

D1 ♀ — Larva (? female) in early phase of 2nd molt.

D2 ♂ — Same, in final part of (?) 2nd molt. D2 ♀ — Larva (female) in final phase of 2nd molt.

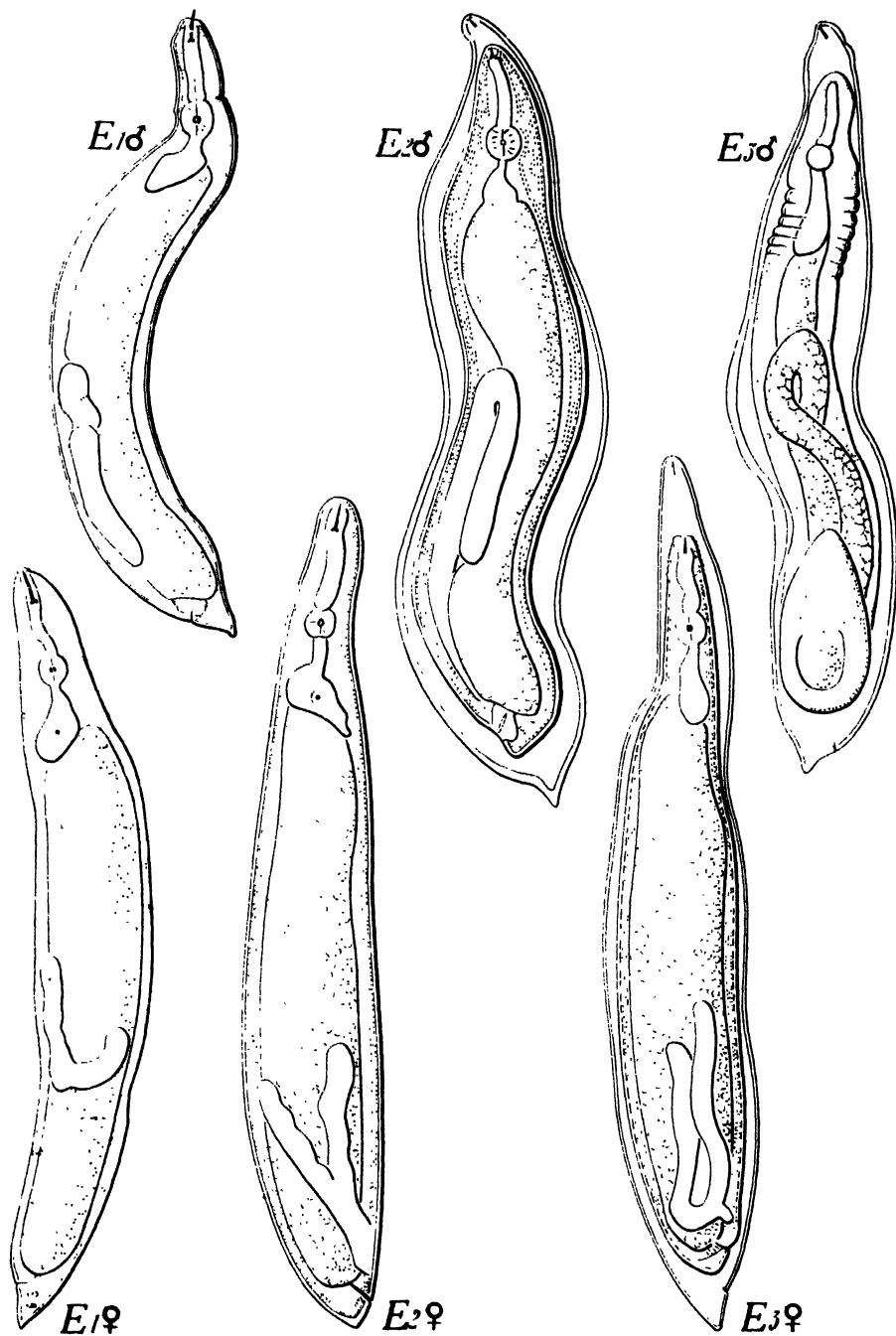


FIG. 2. Stages in the life history of *Heterodera rostochiensis*, continued from figure 1. Lettering and numbering arranged to identify corresponding stages in both sexes. All about 300 \times .

E1♂ — Larva, 3rd stage male.

E2♂ — Larva, molting 3rd stage male.

E3♂ — Larva, coiling early 4th stage male (in 3rd cuticle).

E1♀ — Larva, early 3rd stage female.

E2♀ — Larva, late 3rd stage female.

E3♀ — Larva, molting 3rd stage female.

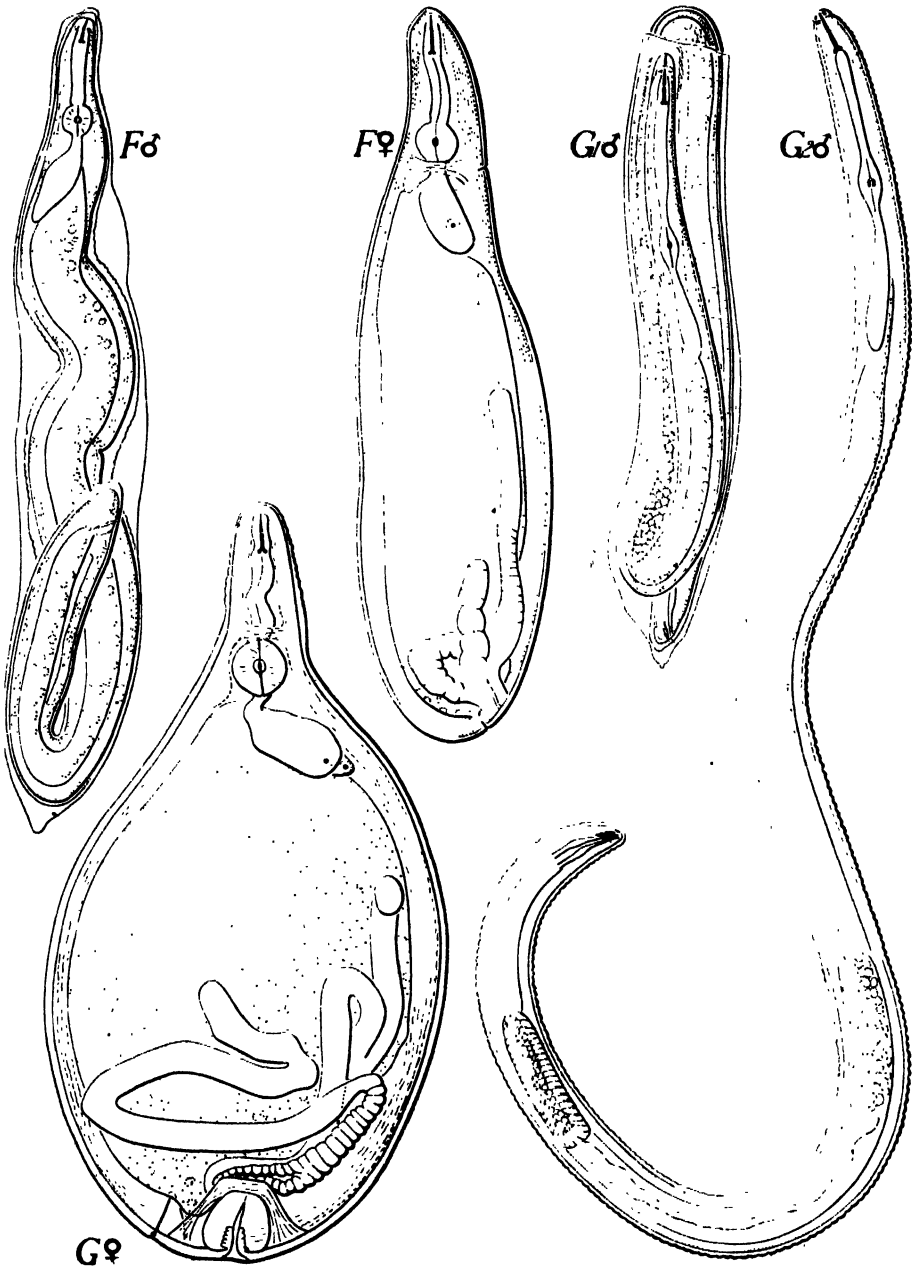


FIG. 3. Stages in the life history of *Heterodera rostochiensis*, continued from figure 2. Lettering and numbering arranged to identify corresponding stages in both sexes. All about 300 \times .

F♂ — Larva, 4th stage male, beginning 4th molt.

G1♂ — Adult male ensheathed in 3rd and 4th cuticles.

G2♂ — Adult male after exsheathing.

F♀ — Larva, 4th stage female.

G♀ — Adult female after 4th molt. (Considerable growth is accomplished after this stage.)

refractive cuticle of the adult female begins to form. Prior to its formation females are practically invisible to the naked eye because of their transparency and protoplasmic gray color. With the advent of the adult cuticle females turn opaque, waxy, or pearly-white, and thus become macroscopically visible. This transformation gives the impression of sudden, multiple emergence from the root, although actually specimens of all stages can be found at this time, if examined microscopically, the posterior portions of their bodies projecting outside the root. Specimens that have completely entered the root may subsequently break through its surface in the third, fourth, or adult stage. Males may either cast or retain the cuticle of the third stage before proceeding to the final or fourth molt.



FIG. 4. Potato roots infected with *Heterodera rostochiensis*. Left: Adult female nematodes shown as spherical bodies clustered on root. $\times 8$. Center: Isolated piece of root showing one female pressing through root surface and several craters where others have fallen out. $\times 4.5$. Right: 2 isolated pieces of root; females glisten immediately after being removed from soil; 7 specimens can be found on extreme right-hand root. $\times 4.5$.

During the 16 days following the final molt the female turns from white to yellow and eggs are produced (Fig. 4). As the season progresses, the females become golden, are finally transformed into a brown cyst containing eggs capable of overwintering, and the cycle is complete. In some instances hatching larvae were observed within yellow females as early as June 16; we have been unable to verify this point, however, from preserved material of that date, although we have seen fully developed larvae present in the eggs. We are therefore uncertain whether such eggs hatch normally, thereby permitting larvae of a second generation to invade potato roots during the same season. It is known that invasion of roots (and tubers) continues throughout the growing season, but this could be the action of larvae from

old cysts. The eggs and larvae from a cyst do not all become active at the same time, but hatch and emigrate over a period of years.

To summarize, then, it may be said that the nematodes pass through the four molts within a period of 23 to 33 days and begin to produce eggs containing larvae within 16 days thereafter. The entire period from embryonated eggs to embryonated eggs is not less than 38 and does not exceed 48 days. During this period, in the experiments of 1944, the weekly mean soil temperatures were 59°, 58°, 63°, 63°, 66°, and 69° F., chronologically.

RELATION TO POTATO HOST

An effort was made to establish a correlation between hatching of eggs plus emigration of larvae, and soil temperatures. Table 1 shows counts of eggs and larvae per cyst on April 25, May 5, and May 26, 1944. There was

TABLE 1.—*Contents of cysts of Heterodera rostochiensis in relation to date, in 1944. (Means of 10 cysts from 10 samples of soil per date)*

Stage of nematode	Number found on		
	April 25	May 5	May 26
Eggs	57	43	43
Larvae	15	15	10
Both eggs and larvae	72	58	53

a definite reduction in the number of eggs in cysts between April 25 and May 5, indicating that hatching was taking place. The number of larvae remained constant between these dates, which we interpret to mean that emigration of larvae from the cysts kept pace with hatching. There is no evidence of hatching between May 5 and May 26, but emigration of larvae continued. Larvae were first seen in potato roots on May 9, in the observations of 1944. Before that date weekly mean soil temperatures had been 49° to 51° F. (between April 7 and 28), then suddenly rose to 59° F. for the week of April 29–May 5. Thereafter they were 58° F. or higher. Evidence in previous years has indicated nematode dormancy at weekly mean temperatures up to 54° F., but there was no such period in 1944.

The life history of the nematode is inalterably linked with that of the potato plant. A study was therefore made of the growth rate of the latter, with special reference to the influence of soil temperature. Potatoes of each of three varieties were planted in five randomized plots on clean and on infested soil, and were allowed to root. After one week, a plant of each variety was taken at random from each of the five clean and five infested plots, and its root system weighed. This process was repeated weekly for the succeeding six weeks. Table 2 gives the total weights of each group of five plants at the various dates, together with the weekly mean soil temperatures of those dates. It will be seen that the variety Irish Cobbler is capable of some root growth at 49° F., while Green Mountain and Houma are not. Both of

TABLE 2.—Weights in grams of root systems of 3 potato varieties

Date (1944)		Variety						Mean soil temperature (degrees F.)
		Irish Cobbler		Green Mountain		Houma		
		Inf. soil	Clean soil	Inf. soil	Clean soil	Inf. soil	Clean soil	
Apr.	25	0.0 ^a	0.0	0.0	0.0	0.0	0.0	49
May	1	0.6	0.1	0.0	0.0	0.0	0.0	49
	8	2.5	4.6	0.1	0.2	0.0	0.4	59
	12	8.1	21.1	0.9	7.0	1.6	14.2	58
	22	23.5	23.4	10.1	18.0	12.6	17.2	63
	29	34.0	42.1	19.2	17.6	33.5	22.0	63
June	5	33.0	41.9	18.0	24.9	45.0	40.3	66

^a Each weight is the total of 5 root systems, 1 plant from each replicate.

the latter are capable of root growth at 58° F.⁴ In comparing the growth in clean and infested soils, unfortunately we cannot attribute the differences solely to the effect of *Heterodera rostochiensis* since other nematode pathogens (*H. marioni* and *Pratylenchus scribneri*) were also present. It can be said, however, that root growth of all varieties was delayed in the infested soil. The difference between root growth in clean versus infested soil was greatest about May 15, after which there was a root stimulation or proliferation in the case of plants growing in infested soil, that tended to reduce this difference.

Observations from previous years had already indicated that *Heterodera rostochiensis* caused minor damage to Irish Cobbler and major damage to Green Mountain and Houma. Injury shows in the form of delayed emergence and stunting of the plants, and reduced yields of the crop. We believe that the relation of spring soil temperatures to nematode activity and to potato growth accounts for the differences in crop losses due to nematodes in the several varieties of potatoes. These relationships are shown in table 3.

TABLE 3.—Effect of soil temperature on growth of potato roots and invasion by *Heterodera rostochiensis*

Mean soil temperature (degrees F.)	Invasion of nematodes	Growth of potato roots ^a	
		Irish Cobbler	Green Mountain
45	—	(+)	—
49	—	+	—
53	(—)	+	?
54	(—)	+	(+)
57	(+)	++	+
58	(+)	++	+
59	+	++	+
62	++	++	++

^a (+) or (—) Activity or inactivity (of nematodes or root growth, as the case may be), as based on circumstantial evidence.

+ or — Activity or inactivity, as based on direct observations of specimens.

++ or -- Pronounced activity or inactivity, as based on direct observations of specimens.

⁴ Evidence from previous seasons indicates minor root growth of Irish Cobbler at 45° F. and of Green Mountain at 54° F.

TABLE 4.—Yields and production costs of 2 potato varieties in clean and in *Heterodera rostochiensis*-infested soils

Variety and year	Yield in cwt./acre			Cost of production (in dollars)/cwt.		
	Clean soil	Mod. inf. soil	Heavily inf. soil	Clean soil	Mod. inf. soil	Heavily inf. soil
Irish Cobbler:						
1941	172		144	0.85		1.05
1942	192		133	0.80		1.15
1943	138	137	70	1.10	1.10	2.15
1944	135	84	82	1.10	1.80	1.85
Summary	135-192	84-137	70-144	0.80-1.10	1.10-1.80	1.05-2.15
Green Mountain:						
1943	148	103	58	1.00	1.45	2.60
1944	110		32	1.35		4.70
Summary	110-148	103	32-58	1.00-1.35	1.45	2.60-4.70

It would seem that Irish Cobbler can grow during the spring when soil temperatures are between 45° and 58° F., before mass root invasion by the nematodes occurs. Green Mountain, however, begins to grow when soil temperatures are 54° to 58° F. Varietal differences in susceptibility to nematode damage are probably correlated with varietal differences in ability to produce roots at temperatures below those at which nematode attack takes place. Differences in injury from year to year within a given variety probably depend upon the duration of mild spring temperatures when the plants can grow free of nematode attack.

To illustrate the importance of this varietal susceptibility we have compared the crop yields and cost of production of Irish Cobbler and Green Mountain on clean, on moderately infested, and on heavily infested soils for the years 1941-1944, where our experimental data were available, together with a summary for those years. On the basis of these figures (Table 4) we may conclude that Irish Cobbler may or may not be profitable on infested land, and that Green Mountain definitely is not profitable.

An effort was made to correlate some potato yields of the entire Nassau County, L. I., N. Y., from 1939 through 1944, with our conclusions. To do so it was necessary to combine losses due to *Heterodera rostochiensis* with those

TABLE 5.—Yields of 2 potato varieties for Nassau County, N. Y., as compared with early spring soil temperatures

Year	Yield in cwt./acre		No. wks. with soil temperature at	
	Irish Cobbler	Green Mountain	45°-58° F.	54°-58° F.
1939	96	73	3	0
1940	191	161	8	4
1941	139	133	5	4
1942	192	144	4	3
1943	141	111	6	2
1944	102	84	5	0

due to *H. marioni*, since temperatures at which the latter invades roots *en masse* according to Godfrey⁵ are nearly identical with temperatures of invasion by the former. Table 5 gives a comparison of the average yields of Irish Cobbler and Green Mountain in Nassau County for these years, together with the number of spring weeks during which the mean soil temperatures there were 45° to 58° F. and 54° to 58° F. The year 1940 was a banner year for potatoes from the standpoints of both county yield and duration of root-growth before nematode attack. On the other hand, the years 1939 and 1944 were characterized by low yields and, significantly, a short period of mild spring temperatures. It seems possible that the length of the period of mild spring weather is a major factor in determining crop production in areas infested with either *H. rostochicnsis* or *H. marioni*.

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⁵ Godfrey, George H. Effect of temperature and moisture on nematode root knot. Jour. Agr. Res. [U.S.] 33: 223-254. 1926.

THE FUNGUS THAT CAUSES SOOTY STRIPE OF SORGHUM SPP.

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The fungus discussed in this paper causes elongate-elliptical lesions on leaves of sorghum and related species (Fig. 1). It occurs in a number of the Southern States, in some cases in considerable abundance.

Larsh (7) in 1944 reported *Septorella sorghi* Ell. and Ev. on sorghum from Oklahoma and Arkansas. Subsequently (8) this identification was corrected to *Titacospora andropogonis* (Miura) Tai, which is the name usually applied to the fungus that causes sooty stripe of sorghum. As the writers were unfamiliar with *Septorella sorghi*, a specimen was requested and material collected in Oklahoma was kindly sent by D. A. Preston. The Oklahoma material agreed perfectly with that collected previously by one of the writers (Lefebvre) at a number of points in several Southern States.

It has been assumed, heretofore, that the first description of the pathogen was that given by Miura (9) in 1920, when he described it on "*Andropogon sorghum* Brot. var. *vulgaris*, subsp. *japonicus* Hack." (*Andropogon sorghum* (L.) Brot. subsp. *sativus* Hack. var. *vulgaris* Hack. subvar. *japonicus* Hack. = *Sorghum vulgare* Pers.). Miura created a new genus for the fungus and called it *Ramulispora andropogonis*. He placed the genus in a new subfamily of the Melanconiales. Tai (11), in 1932, transferred the species to the previously described genus *Titacospora* Bubák (4), another genus of the Melanconiales, making the name *T. andropogonis* (Miura) Tai. This is the name by which the fungus had been known up to 1944 when Larsh (7) reported ~~on~~ *Septorella sorghi*.

In 1903, Ellis and Everhart (5) described *Septorella sorghi* on the leaves of *Sorghum halepense* (L.) Pers. collected by George W. Carver at Tuskegee, Alabama, in September, 1901. A part of the type collection of this fungus was kindly supplied by J. C. Gilman from the herbarium of Iowa State College. An examination of the fungus showed that it was identical with the one described by Miura (9). Thus, *S. sorghi* Ell. and Ev. antedates *Ramulispora andropogonis* Miura by 17 years. Ellis and Everhart, however, had mistaken the sclerotia for pycnidia, and apparently had not noticed that the conidia were branched.

In two recent papers, Bain (2) and Bain and Edgerton (3) have pointed out that the fungus has been incorrectly classified and that the fruiting body

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FIG. 1. Lesions of the sooty stripe disease on sorghum. A and B, Lesions on sorghum leaves collected in the field; C, Lesions produced by inoculation in the greenhouse; D, Lesion produced by inoculation, showing sclerotia; E, Part of the same lesion as D, enlarged.

actually is more like a sporodochium than an acervulus. The studies by the writers agree with this.

In an attempt to determine the relationship of *Septorella sorghi* and *Tipecospora andropogonis* and to establish the systematic position of these fungi in the Fungi Imperfecti, all of the above-mentioned material and other herbarium specimens were studied, as well as fresh material from plants artificially inoculated in the greenhouse. Studies were made also to obtain a better knowledge of the life history and parasitism of the fungus that causes the sooty stripe disease.

DISTRIBUTION OF THE FUNGUS

The fungus has been found on the following hosts in the States indicated. The reports not credited are based on specimens collected by one of the writers (Lefebvre).

On *Sorghum halepense* (L.) Pers., Alabama (5), North Carolina, Mississippi; on *S. vulgare* Pers., Alabama, Arkansas (7), Florida, Georgia, Louisiana (1), Mississippi (10), Oklahoma (7); on *S. halepense* \times *S. vulgare*, Mississippi; on *S. vulgare* var. *sudanense* (Piper) Hitchc., Florida, Georgia, Texas; on *S. vulgare* \times *S. vulgare* var. *sudanense*, Georgia.

MORPHOLOGY OF THE FUNGUS

The mycelium, stromata, sporodochia, conidiophores, conidia, and sclerotia have been studied chiefly on field and greenhouse material of sorghum in variously stained free-hand and microtome sections of lesions. Phloxine in Patterson's mounting fluid has been found satisfactory for hand sections, and safranin and fast green for microtome sections. Unstained dried and fresh material also has been studied.

The hyphae are chiefly intercellular in the parenchyma of the leaf tissue (Fig. 2, C) but are intracellular in the vessels (Fig. 3, C' and D). In the vessels, the hyphae extend longitudinally in the leaf, thereby lengthening the lesion. Eventually the hyphae tend to aggregate just beneath the stomata to form more or less compact stromata from which the conidiophores arise (Fig. 2, B and C', and Fig. 4, B). Frequently, the substomatal hyphae, and the conidiophores arising from them, are few and not aggregated into compact masses (Fig. 2, A, and Fig. 4, A). Typically, the conidiophores mass together into a column, which eventually collapses the guard cells (Fig. 2, C, and Fig. 3, A). These conidiophores usually extend to a point somewhat above the epidermis and bear the conidia singly at their tips, and the conidia eventually become aggregated into gelatinous masses (Fig. 4, C). With temperature and moisture favorable for the fungus, the fructifications continue to develop and expand. The stromata, as well as the conidiophores, eventually become sclerotized (Fig. 3, A, and Fig. 4, D). Conidia continue to be produced until all of the conidiophores become sclerotized. The entire fructification may be converted into a black sclerotium which ceases to produce conidia (Fig. 4, E, a). Thus the fruiting structure without question is to be interpreted as a sporodochium.

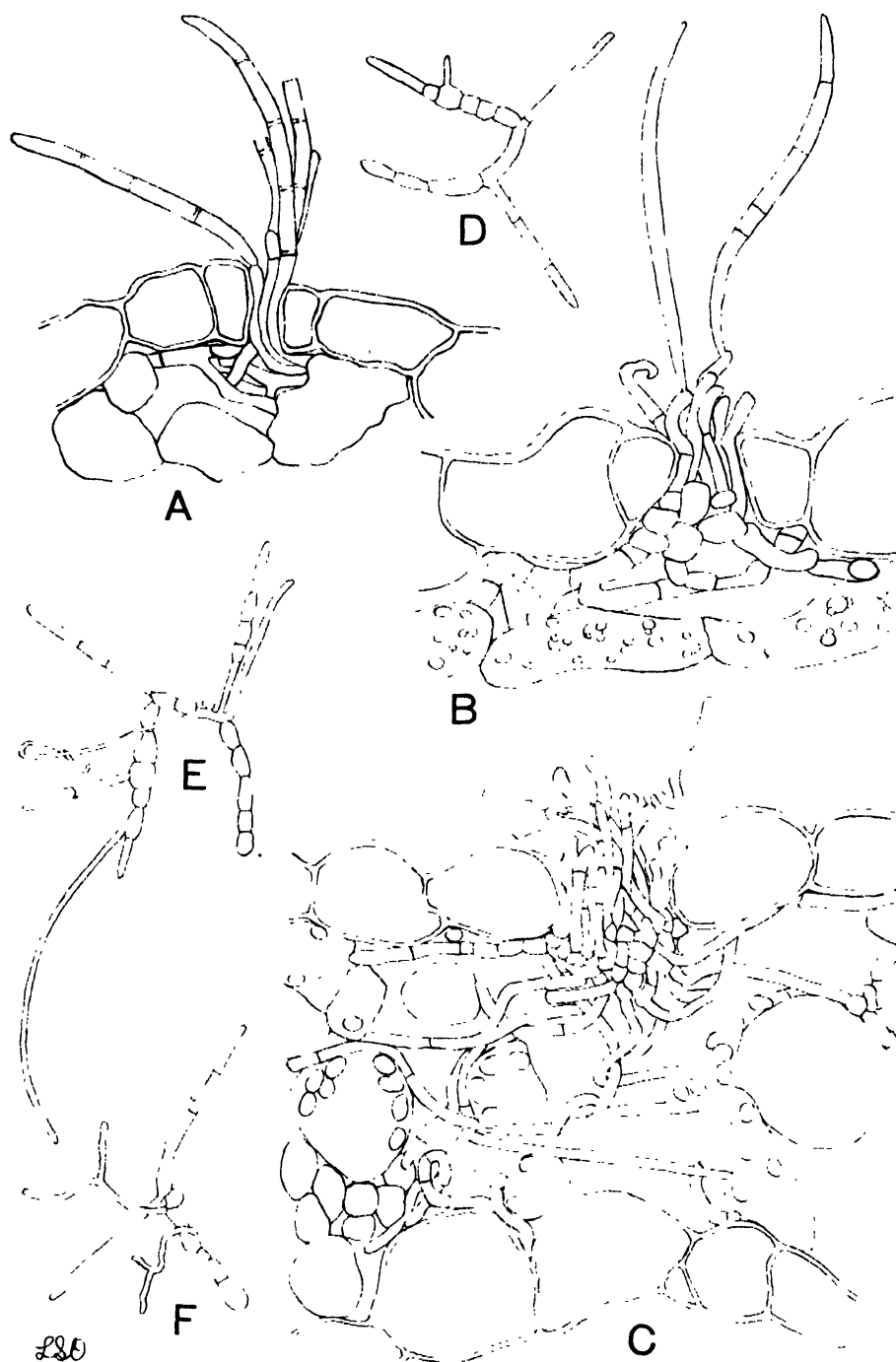


FIG. 2. *Ramulispora sorghi*. A, Small conidial fructification in stoma (Same as Fig. 4, A); B and C, Young sporodochia in stomata (C also shown in Fig. 4, B); D to F, Conidia germinating on agar. (A to C, $\times 945$; D to F, $\times 400$.)

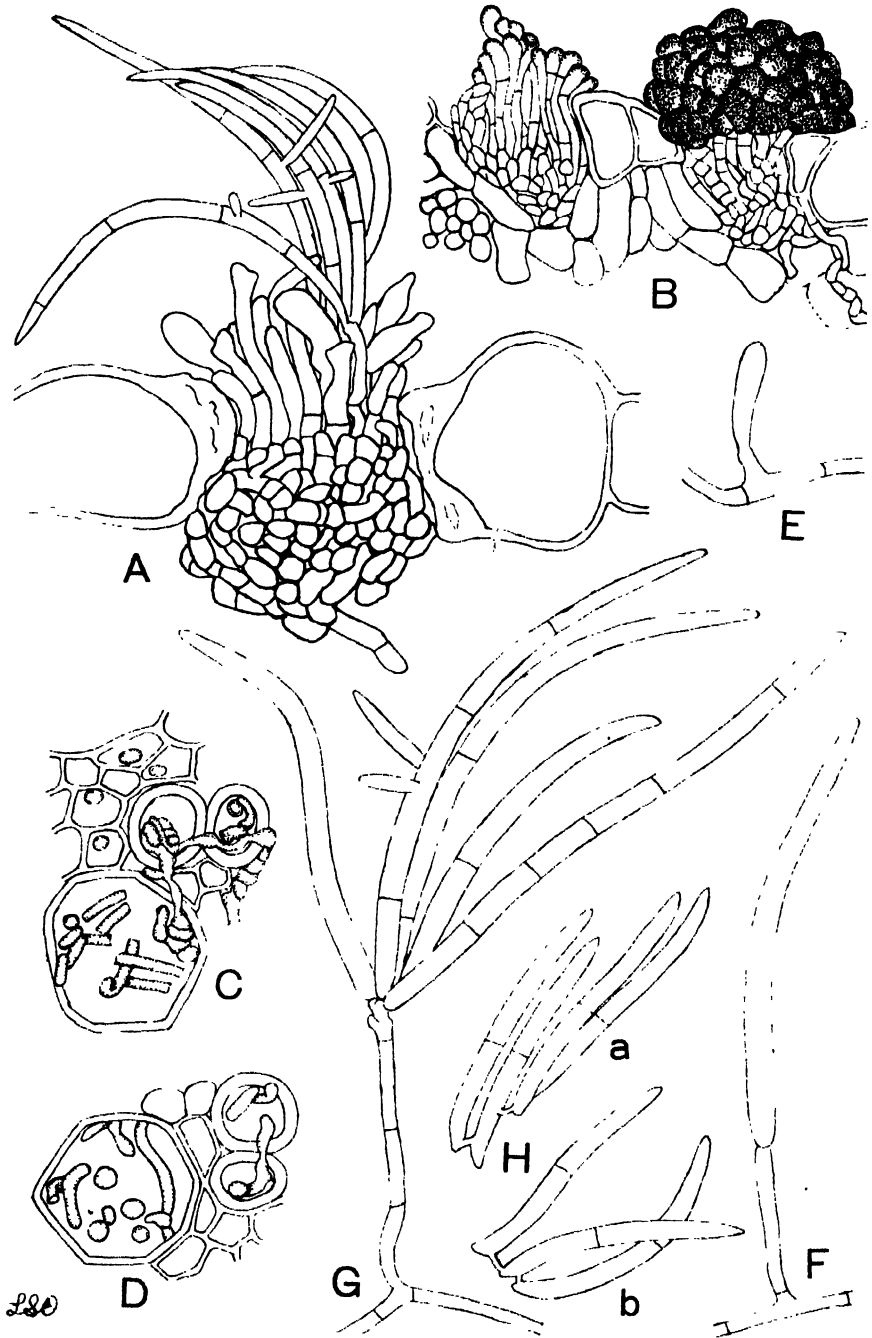


FIG. 3. A to G, *Ramulispora sorghi* on sorghum; A, Sporodochium, showing sub-stomatal stroma, conidiophores, and branched conidia; B, Developing sclerotia; C and D, Hyphae in the vessels; E to G, Conidiophores and conidia as they appear inside the agar medium; H, a and b, Fused conidia of *Tilacospora detospora*. (All figures $\times 945$, except B, $\times 450$.)

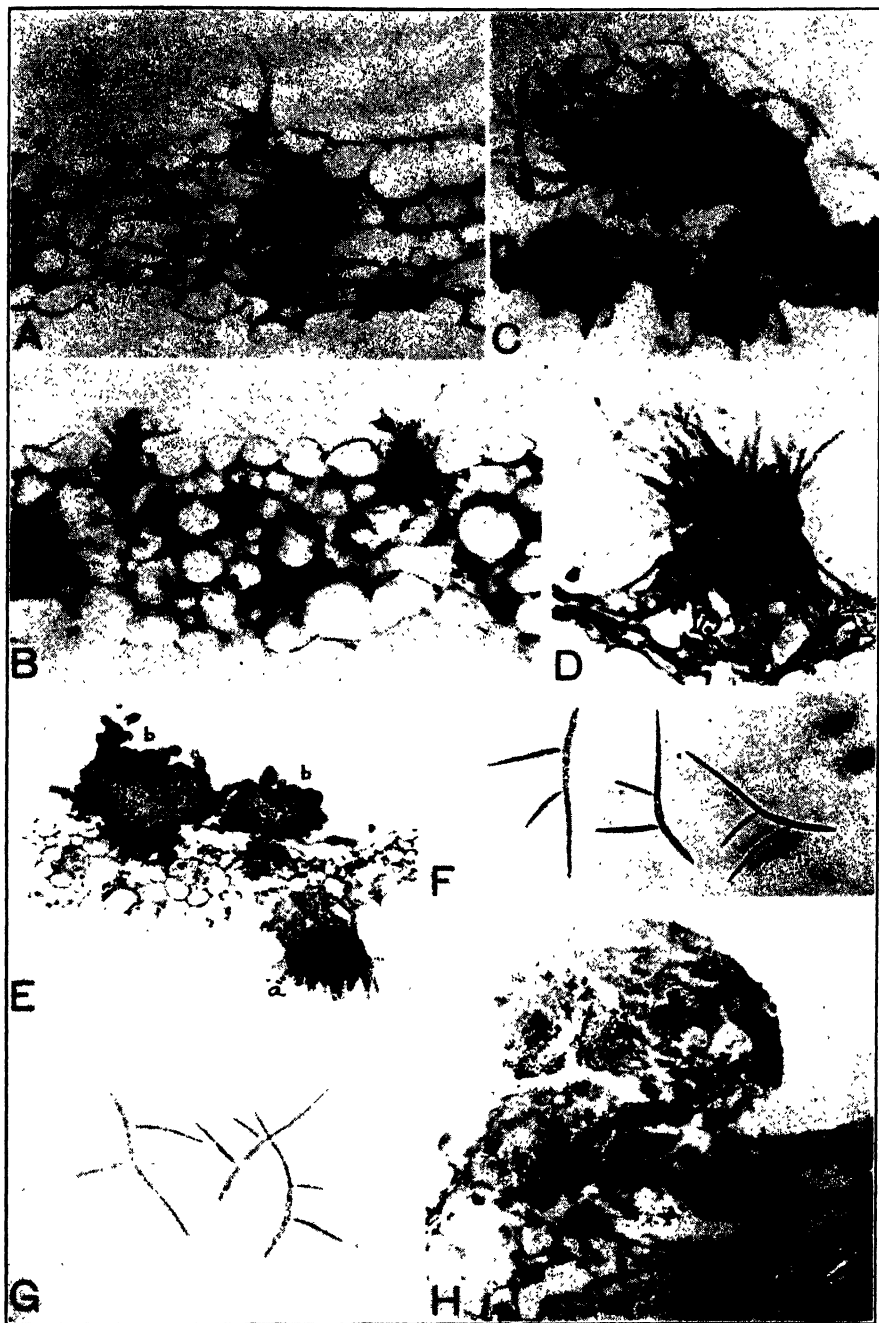


FIG. 4. Photomicrographs. A to G, *Ramulispora sorghi* on sorghum; H, *Titacospora detospora* on *Equisetum*; A, Small conidial fructification; B, Two young sporodochia; C, Sporodochium with mass of conidia; D, Mature, partly sclerotized sporodochium with conidia; E, Sclerotized sporodochium (a) and two sclerotia (b); F and G, Conidia; H, Acervulus of *Titacospora detospora* with exuding mass of conidia. (All figures $\times 400$, except E, $\times 155$, and H, $\times 90$.)

The conidia are long and slender, hyaline, curved, tapering gradually toward the apex, $38-86.3 \times 1.9-3 \mu$. They become 3- to 8-septate and produce 1 to 3, usually 2, lateral branches, which become 0- to 3-septate and measure $5-53 \times 1.1-2.5 \mu$ (Fig. 3, A, and Fig. 4, F and G). When the conidia are germinated on agar, their cells swell, often appear chlamydospore-like, and send out narrow germ tubes (Fig. 2, D to F).

Most of the large black sclerotia that appear on the surface of the lesion seem to arise independently of the sporodochia, although it is not always possible to distinguish between sclerotized sporodochia and young sclerotia. If diseased plants are kept in an environment unfavorable for conidial production, only sclerotia appear, and many of these seem to develop from what would have been sporodochia. In fact, if the lesions are placed in a moist chamber at this stage, some of the immature sclerotia produce conidia very readily. After the sclerotia have matured fully, however, they usually cannot be induced to produce conidia.

In descriptions from the Orient, the sclerotia have been described as occurring only in autumn (9, 11). In a collection sent by M. L. Lohman from Mississippi, they were produced as early as June 11. Other records show that in the United States they appear throughout the summer and autumn. Their occurrence is dependent, not so much on the time of the year, as on the condition of the dying, diseased tissue.

The sclerotia, like the sporodochia, arise from the sub-stomatal stomata that give rise to columns of hyphae that pass through the stomata and form hemispherical masses of enlarged hyphal cells on the surface of the leaf (Fig. 3, B, and Fig. 4, E, b). The entire structure becomes sclerotic. At maturity, the hemispherical bodies are brittle and easily broken off and dislodged from the leaf surface. These sclerotia are hard, black, and tuberculate and measure $53-170 \mu$ in diameter.

In order to determine how the fungus overwinters and how infection occurs the following spring, some heavily infected leaves that were covered with sclerotia were placed outdoors and left there through the winter of 1944-45 at the Plant Industry Station, Beltsville, Maryland. On May 22, 1945, some of the material was brought in and examined microscopically. The sclerotia were unchanged and live conidia were almost completely lacking. Some of the leaf fragments with numerous sclerotia on them were placed on moist blotting paper in a Petri dish and left overnight. The following day, many of the sclerotia were producing conidia in great profusion. Gelatinous aggregates of conidia, like those already described, were observed. Free-hand-sections of the material showed that the conidia were being produced chiefly at the top of the sclerotium where a group of conidiophores had appeared. Although the conidia are produced entirely outside the sclerotium, the latter appears, superficially, like a pycnidium with conidia exuding from an ostiole. Some conidia were observed arising from sclerotia that had been arrested early in their development. Thus it appears that the sclerotia function primarily in overwintering the fungus and that the conidia produced by them are the chief source of infection in the spring.

CULTURAL CHARACTERISTICS OF THE FUNGUS

The fungus was grown on various culture media including malt, potato-dextrose, prune, corn-meal, bean-pod, and sorghum-leaf agars. In all cases, growth was rather slow. On all agar media, the fungus grew in raised, black, tough, tuberculate masses, appearing somewhat like an aggregation of sclerotia. Several weeks were required for good conidial production. Conidia, similar to those on leaf lesions, were produced abundantly on short conidiophores in light pink, gelatinous, cone-shaped masses all over the rough surface of the black fungal cushions on all agars, except malt agar, on which very few of them appeared.

In agar cultures, a considerable part of the fungal growth was beneath the surface of the agar. In thin sections cut through the interior of the agar, small black specks were observed all through the medium. An examination of these under a microscope showed that they were small aggregates of swollen hyphal cells. Furthermore, the fungus was growing throughout the interior of the agar medium and sporulating there like a moniliaceous fungus. One to several conidia were produced on conidiophores of various lengths, which arose from hyaline hyphae throughout the medium (Fig. 3, E to G). The conidia were typical in appearance, except that usually they were larger than those produced on the sclerotia on the surface of the agar.

Other culture media used were carrot juice plus 1 per cent dextrose, sterilized stems of sorghum and Johnson grass, cowpea pods, and string beans. Again the fungus grew slowly in the form of conspicuous, black, tuberculate masses. Conidia were scarce on all of these media except in the carrot-juice concoction, in which they were produced in abundance.

An effort was made to find the perfect stage in culture, in material from the field, and in overwintered leaves, but without success.

TAXONOMY OF THE FUNGUS

The foregoing investigations have shown that the conidial fructification of the fungus that causes sooty stripe of sorghum, Sudan grass, and Johnson grass is a sporodochium and not an acervulus. This fungus, in some respects, is like *Gloeocercospora sorghi*, recently described by Bain and Edgerton (3) on sorghum. Both fungi have sporodochia and sclerotia; but, as noted above, Ellis and Everhart (5) named the fungus *Septorella sorghi* in 1903, thinking that the fruiting structure was a pycnidium. In 1920, Miura (9) established the genus *Ramulispora* and described this fungus as *R. andropogonis*. In 1932, Tai (11) transferred the species to the genus *Titacospora* Bubák, making the combination *T. andropogonis* (Miura) Tai.

The genus *Titacospora* Bubák, which has *T. detospora* (Sacc.) Bubák on *Equisetum* as the type species, has true acervuli (Fig. 4, H). Bubák (4) characterized the genus chiefly by branched conidia produced in acervuli. Von Höhnelt (6), however, pointed out that what Bubák had taken for branched conidia in *T. detospora* in reality were not branched conidia but were conidia that were fused at or near their bases. The writers examined

authentic material of *T. detospora* on *Equisetum* and found the same type of conidial fusion as described by Von Höhnelt (Fig. 3, H, a and b). This being the case, the fungus that causes sooty stripe cannot go into the genus *Titacospora*, because its fruiting structure is a sporodochium and it has characteristically branched spores.

Miura (9) described the genus *Ramulispora* as follows:

“*Melanconiales*

“*Melanconiales*—*Hyaloramulisporeae*. M. Miura, N. Subf. *Ramulisporea* M. Miura, n. g. Spores cylindrical, flexuose, slender, branched, septate, other characteristics as in the case of *Cylindrosporium*.”

To be sure, Miura interpreted the fruiting structure as an acervulus, but the other characters, including the branched conidia, agree perfectly with those of the fungus studied by the writers. The writers, therefore, propose that the generic name *Ramulispora* be retained, but that it be transferred to the Tuberculariaceae, thereby abolishing Miura's sub-family, and that the description of *Ramulispora* be emended as follows:

Sporodochia amphigenous, produced through stomata of infected leaves, arising from sub-stomatal stomata; conidiophores hyaline, simple or branched, short; conidia acrogenous, hyaline, filiform, with lateral branches, produced in gelatinous aggregates; superficial sclerotia present.

Since *Septorella sorghi* Ell. and Ev. is the oldest specific name of the fungus, the following is proposed:

***Ramulispora sorghi* (Ell. and Ev.) Olive and Lefebvre, Comb. Nov.**

Septorella sorghi Ell. and Ev. (5) 1903.

Ramulispora andropogonis Miura (9) 1920.

Titacospora andropogonis Tai (11) 1932.

Spots elongate-elliptical, with straw-color centers, surrounded by reddish-purple to tan borders according to the variety. Sclerotia amphigenous, gregarious, superficial on straw-color centers of lesions, sub-globose, coarsely tuberculate, subcarbonaceous, 53–170 μ ; sporodochia, amphigenous, develop from sub-epidermal stomata, becoming crumpled through stomata; conidiophores fasciculate, 10–35 \times 2–3 μ ; conidia filiform, with 1 to 3 branches, 5–53 \times 1.1–2.5 μ , hyaline, curved, tapering toward apex 38–86.3 \times 1.9–3 μ 3- to 8-septate.

On *Sorghum halepense* (L.) Pers.—Alabama, North Carolina, Mississippi.

On *S. vulgare* Pers.—Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, Oklahoma.

On *S. halepense* \times *S. vulgare*—Mississippi.

On *S. vulgare* var. *sudanense* (Piper) Hitchc.—Florida, Georgia, Texas.

On *S. vulgare* / *S. vulgare* var. *sudanense*—Georgia.

In the original description of the fungus, Miura (9) gave 36–100 \times 2–4 μ as the spore measurements, and Tai (11) gave 47.6–106.9 \times 2.04–3.06 μ . While these length measurements are somewhat greater than those obtained by the writers, this difference is not considered significant, as the length of spores of this type have a wide range, being influenced greatly by the environment in which they are produced.

PATHOGENICITY OF THE FUNGUS

Typical leaf spots produced by this fungus in the field begin as small, oblong, reddish-purple spots, which develop into conspicuous elongate lesions

with purplish borders and straw-color centers of dead tissue. This dead tissue usually is more or less densely covered with small black sclerotia, which may impart a sooty appearance to the lesion (Fig. 1, A and B), hence the common name sooty stripe given to the disease by Bain (2). The spots are about equally distinct on both sides of the leaf.

In some varieties of sorghum, such as Leoti, and in Tift and Sweet Sudan grass, in which the purple leaf pigment is absent, the lesions have a tan instead of the purple border described above; and on Johnson grass the purple border usually is not so pronounced as in most varieties of sorghum. Mature spots, however, are easily recognized by the numerous sclerotia on the surface of the lesions.

If the lesions are examined with a binocular microscope or even with a hand lens after they have been taken from a warm, moist environment, numerous cones of agglutinated, flesh-color masses of conidia can be seen arising from either leaf surface. Sclerotia in various stages of development also may be scattered over the same area. Both the sclerotia and conidia appear to be somewhat more abundant on the lower than on the upper side of the leaf.

In a nursery of sorghum varieties, kindly arranged for in 1944 by J. D. Warner at the North Florida Experiment Station, Quincy, Florida, very light to moderate infections by *Ramulispora sorghi* were observed on the following varieties: Rex, Planter, Colman, Saccaline, Leoti, Denton, Rox Orange, Sapling, Brown Durra, Norkan, Atlas, Silver Top, and Gooseneck.

To determine whether symptoms similar to those found in the field could be produced in the greenhouse, plants of sorghum and related species were inoculated in the spring of 1945 by spraying four or five times over a period of two days with aqueous suspensions of conidia and hyphae made from cultures of *Ramulispora sorghi* grown on agar or in carrot juice. The latter medium was the most effective in producing large amounts of inoculum.

Three series of inoculations were made. Within a few days after each series had been inoculated, small, scattered, purplish spots appeared on the leaves, but no conspicuous lesions were observed until about 16 to 18 days after inoculations had been made. During this period, the plants were left in the moist chamber most of the time. The lesions were mostly like those observed in the field, although usually they were not so large (Fig. 1, C). When plants with mature lesions were returned to the moist chamber, conidia appeared on the lesions in great abundance. At the same time sclerotia also were readily produced (Fig. 1, D and E). Apparently they were more abundant on plants kept in the moist chamber at this stage, although they developed also on those kept outside the moist chamber.

The relative susceptibility of the sorghum varieties and related species inoculated with *Ramulispora sorghi* may be given tentatively as follows. An accurate interpretation of results, however, was not possible in every case because many leaves on the test plants died or developed streaks before typical lesions were produced. Heavy infection was obtained on Brown Durra; on Orange, Rex, and Sugar Drip sorgho; on White kaoliang; on common and

Tift Sudan grass; and on Johnson grass. Moderate infection occurred on Texas Blackbull kafir; on Saccaline, Minnesota Amber, and on Hodo sorgho; on Standard broomcorn; on Dwarf Yellow and Martin milo; and on Sweet Sudan grass. Light infections resulted from inoculations carried out on Honey, Early Folger, Atlas, Iceberg, and Leoti sorgho; and on Plainsman milo, Spur feterita, and Early Hegari varieties of sorghum. Wherever the large conspicuous lesions appeared, they developed the typical straw-color centers and purple borders, except in varieties where the purple pigment was absent in the leaves.

SUMMARY

The morphology of the fungus that causes sooty stripe of sorghum, Sudan grass, and Johnson grass is described. Mature lesions caused by the fungus usually are covered by numerous black sclerotia, have a straw-color center and are surrounded by a conspicuous purple border. In a moist atmosphere, sporodochia push through the stomata and produce conidia in light pink, gelatinous masses. These conidia are filamentous, curved, and septate, with 1 to 3 lateral branches. Small, black, tuberculate sclerotia also are produced in large numbers through the stomata. The fungus overwinters by means of these sclerotia, which germinate to produce conidia in the spring.

The fungus may be cultured on most artificial media, but develops very slowly. In culture, it grows in the form of raised tuberculate, black masses, on which the conidia appear in light pink, gelatinous aggregates. Inside the agar medium the organism grows and sporulates like a moniliaceous fungus.

Typical lesions with sclerotia, sporodochia, and conidia have been produced readily by inoculating several varieties of sorghum, Johnson grass, and Sudan grass in the greenhouse.

The genus *Ramulispora* is emended and transferred from the Melanconiales to the Tuberculariales of the Fungi Imperfecti and the new combination *Ramulispora sorghi* is made.

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AN UNDESCRIBED EAR ROT OF CORN CAUSED BY PHYSALOSPORA ZEAE¹

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INTRODUCTION

For the past several years an ear rot of corn (*Zea mays* L.) has been found in Indiana that could not be identified with any of the known diseases of corn ears. During the period that the disease has been under observation it never has been found in great abundance. In 1944, however, its prevalence reached as high as 10 per cent in some localities in the east-central and southern areas of the State.

The causal fungus remained unidentified for a number of years because it did not produce fruiting structures either on infected ears in the field or in pure culture. Recently it was induced to sporulate on certain media, thereby facilitating its proper identification.

The present paper describes the symptoms of the disease as it affects ears of corn and reports experiments to establish identity of the pathogen.

SYMPTOMS

The first indication of infection is premature bleaching of the husks of the ear. As the disease progresses the fungus mycelium cements the husks to each other and to the kernels. At this stage, the more or less felty, white mycelium is observable on and between the kernels (Fig. 1, A) and, when the ear is broken, the pith of the cob has a characteristic slate-gray discoloration and is dotted with black sclerotia (Fig. 1, H).

As the disease continues to develop, mycelium grows between the husks, over the kernels, and within the cob. The white felty layer of mycelium between the inner husks soon shows irregular blotches and streaks of gray, particularly near the base of the ear (Fig. 1, B). At this stage, when the infection is well developed, the kernels often are speckled because of the formation of black sclerotia beneath the pericarps (Fig. 1, C, D, E). Heavily infected kernels often are black at the tip, the coloration extending for varying distances toward the crown. When ears are kept moist for a long time, as may occur in rainy weather or when the ear is in contact with the ground, or when the ear contains a high percentage of natural moisture, the seed coat has black streaks (Fig. 1, F). Sections through badly infected kernels show the embryo to be completely overrun by black mycelium of the fungus. Beneath the seed coats of such kernels black stromatic layers of

¹Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, and the Department of Botany and Plant Pathology, Purdue University Agricultural Experiment Station. Journal Paper No. 200 of the Purdue University Agricultural Experiment Station.

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mycelium are frequently seen (Fig. 1, G). There is some shredding of the cob at the base of the ear, but this is not so extensive as in *Nigrospora* cob rot (Fig. 1, I). Completely rotted ears have dark gray husks, and cobs and kernels are reduced to a dry gray-black pithy mass (Fig. 1, B).

Infection takes place generally near or through the butt of the ear, but occasionally ears are found where infection was initiated through the tip.

In many respects this ear rot closely resembles *Diplodia* ear rot, particularly in initial phases of its development (Fig. 1, A), and the two diseases may easily be confused if superficially observed. The slate-gray color and the presence of black sclerotia in the cob are symptoms distinguishing this disease from *Diplodia* ear rot in relatively early stages. The gray mycelium

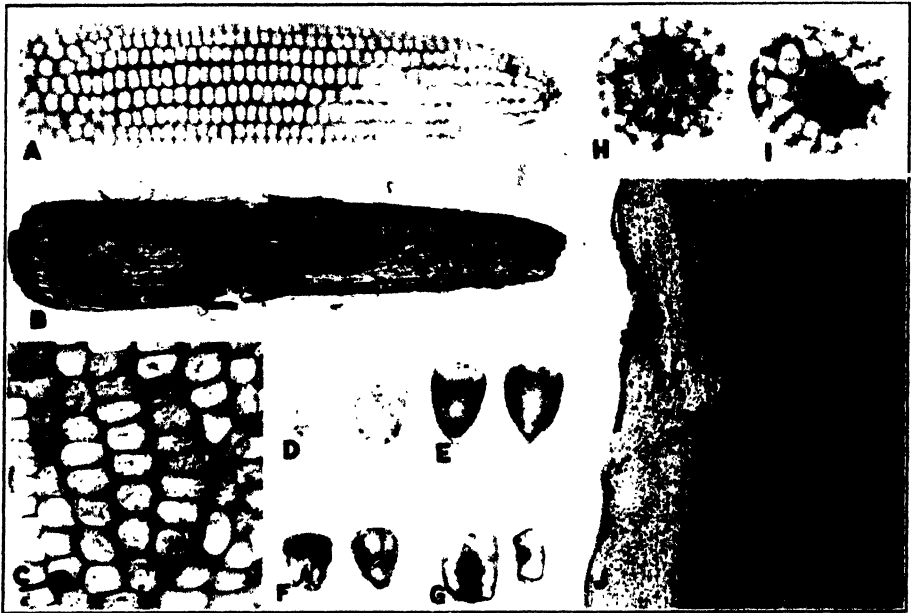


FIG. 1. Typical symptoms produced by the gray-ear rot fungus. A. Early stage of infection; note similarity in appearance to *Diplodia* ear rot. B. Advanced stage of the disease, with blotches of gray mycelium between the husks. C, D, E. Speckled crowns and faces of kernels due to sclerotia beneath pericarps. F. Kernels with black striations. G. Badly infected kernel, showing gray-black coloration of the germ and beneath pericarp. H. Cross section through an infected ear, showing development of sclerotia. I. Base of a diseased ear, showing the shredded end of the cob. J. Typical lesion on corn leaf caused by *Physalospora zeae*.

between the husks and the black sclerotia beneath the pericarps of the kernels are features that further differentiate it. In advanced stages, the gray-black color and dry pithiness of completely rotted ears are distinct from symptoms of *Diplodia* ear rot.

In order to avoid confusion with a *Physalospora* ear rot occurring in Florida and caused by *Physalospora zicola* Ell. and Ev., the common name "gray ear rot" is suggested. The name, gray ear rot, is descriptive of the distinctive gray color of the husks and the appearance of the interior of the cob.

ATTEMPTS TO INDUCE THE FUNGUS TO SPORULATE

The ear-rot fungus was grown on a variety of vegetable extract agars including potato-dextrose, corn-meal, malt-extract, oatmeal, Lima-bean, prune, corn-leaf-decoction, and carrot-extract. Plates of each substrate were divided into two groups, one of which was held in the dark, the other in diffuse light. Cultures were incubated in a temperature range of 20° to 24° C. The fungus was also grown on synthetic agars in which the carbon and the nitrogen sources were varied both qualitatively and quantitatively.

With the exception of malt-extract agar, none of these media supported development of any fruiting structures. On malt-extract agar small botryoid masses were found on old cultures that had been incubated for 6 weeks. These masses were carbonaceous clusters of black pycnidium-like bodies bearing minute, elliptical to oblong, hyaline spores that failed to germinate.

A number of investigators (4, 7) have demonstrated the production of fruiting structures by certain fungi when stimulated by the presence of other fungi or bacteria, or their extracts. Accordingly cultures of the gray-ear-rot fungus were seeded on corn-meal-extract, oatmeal, potato-dextrose, and malt-extract agar in direct association with *Diplodia zae* (Schw.) Lev., *Nigrospora oryzae* (Berk. and Br.) Petch, *Gibberella zae* (Schw.) Petch, *Gibberella fujikuroi* (Saw.) Wt., and *Helminthosporium turcicum* Pass. In another experiment these same fungi were grown in liquid culture on malt extract, potato-dextrose extract, corn-meal extract, and Czapek's medium. After 14 days these staled media were sterilized by passing through Seitz filters, added to liquefied agar, poured in Petri dishes, and seeded with the gray-ear-rot fungus. In both experiments the cultures were incubated in diffuse light at room temperature (21° to 24° C.) for 6 weeks. Sporulation failed to occur either in direct association with or on media staled by other fungi.

Natural substrates, including whole oats, whole wheat, whole corn, polished rice, corn meal, oat straw, soybean stems, carrot, green bean, and potato tuber plugs, were sterilized and seeded with the ear-rot fungus. These cultures were incubated in diffuse light at room temperature. No fruiting structures were produced on any of these media, with the exception of soybean stems. On the soybean stems the pycnidium-like bodies, mentioned as occurring on aged malt-extract agar and bearing minute nongerminating spores, were sparsely produced.

Young cultures of the gray-ear-rot fungus growing on potato-dextrose agar were irradiated with ultraviolet light provided by a Cooper-Hewitt 4 amp., 900 watt, D.C. lamp. The cultures were held 35 cm. from the lamp and exposed, without filtration, from 10 to 240 seconds. Hyphal tip transfers from the margins of the colonies treated for 10 to 180 seconds grew normally. Transfers from colonies irradiated for 210 seconds ceased to grow 24 hours after having been implanted on fresh agar. Transfers from cultures exposed 240 seconds failed to grow. In none of the surviving cultures did sporulation take place.

Kernels removed from diseased ears were held frozen in ice cubes for 2 days to 4 weeks, after which they were removed and planted in moist chambers or surface sterilized and plated on agar substrates. Portions of diseased ears were partially buried in sand in the greenhouse. Some of these were kept constantly wet, others were alternately moistened and dried. Infected ears were placed on the surface of the soil out of doors for several months during the winter and examined in the spring. None of these treatments of diseased host material stimulated formation of fruiting bodies.

In view of the negative results obtained in attempts to induce sporulation, so as to facilitate identification of the gray-ear-rot fungus, intensive studies on this phase of the problem were discontinued until further leads from field observations might come to light. Although fruiting structures were produced in limited numbers on malt-extract agar and soybean stems, the failure of the spores to germinate precluded completion of the life cycle of the fungus.

THE OCCURRENCE OF *PHYSALOSPORA ZEA* STOUT IN INDIANA

In September, 1943, leaves of corn bearing relatively large lesions were found in widely separated localities in the State. These lesions were tan to grayish brown and ranged from 1 × 2 inches to 4 × 18 inches. Scattered over the surfaces of the lesions were numerous small, black, erumpent structures suggestive of some type of fruiting body (Fig. 1, J). In several fields examined the tassels and flag leaves of some plants were bleached out and the tassel necks, or uppermost internodes immediately below the tassel, were inclined at an angle. The bleached condition of these parts stood out in marked contrast to the green leaves and upright tassels of healthy plants. Some elliptical lesions on the tassel necks were water-soaked while others were dry and gray-brown. Black structures similar to those on the leaf lesions, were scattered over the surfaces of dry lesions on the tassel necks.

Examination in the laboratory showed these fruiting structures to be immature, but after 7 to 10 days in a moist chamber, pycnidia and perithecia matured. These fruiting structures were tentatively diagnosed as *Macrophoma zea* Tehon and Daniels, and *Physalospora zea* Stout. Comparisons with the type specimens of these fungi definitely proved the identity of the Indiana collections.³ So far as can be determined this is the first record of these fungi in Indiana.

In the lesions on tassel necks were a few pycnidium-like bodies bearing very small hyaline, single-cell, non-germinating spores. These bodies were similar to the structures that appeared sparsely in certain pure cultures of the gray-ear-rot fungus. Their presence on host material infected with *Physalospora zea* and also in pure cultures of the fungus, suggested that *P. zea* and the gray-ear-rot pathogen might be related.

Isolates derived from single pycnidiospores of *Macrophoma zea* and from single ascospores of *Physalospora zea* were strikingly similar, in cultural

³ The writer is indebted to Dr. L. R. Tehon for the privilege of examining collections of this material in the herbarium of the Illinois Natural History Survey.

behavior, to each other and also to isolates of the gray-ear-rot fungus. Further observations on the similarity in colony characteristics and growth rate between *M. zeae*, *P. zeae*, and the ear-rot fungus were made on a number of agar substrates. On all the media used, no differences among the three isolates were evident. The characteristic growth habits of the three isolates

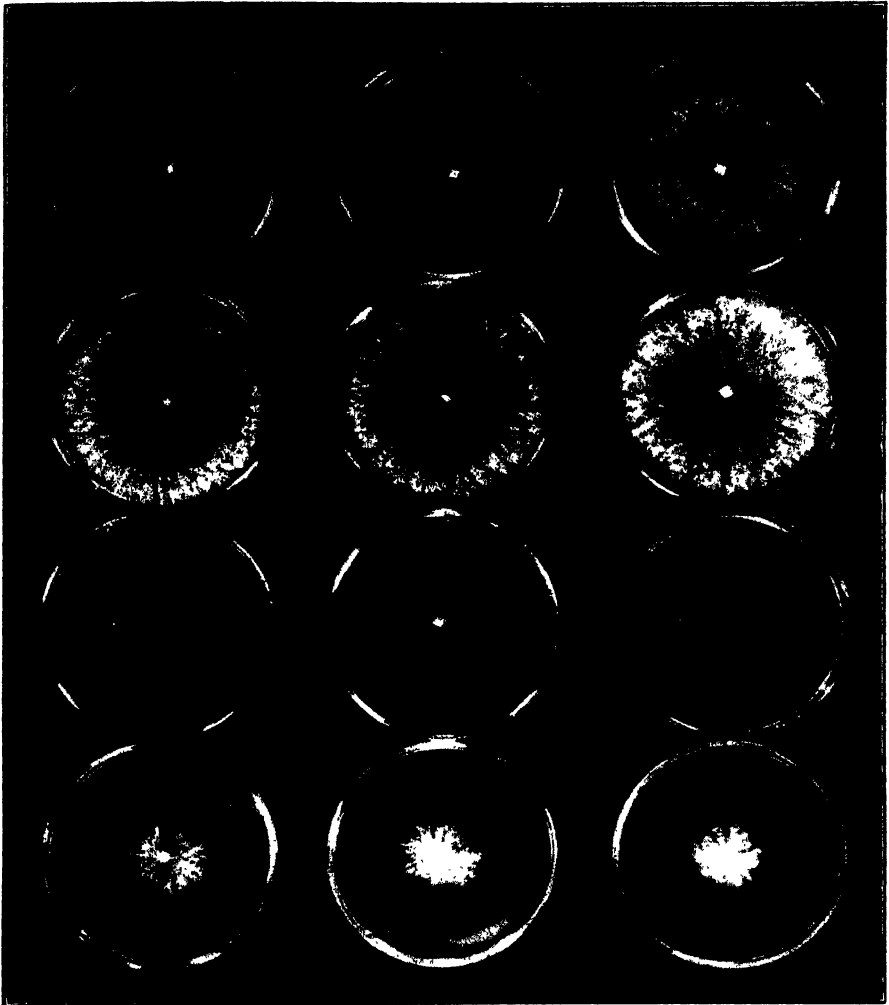


FIG. 2. Comparison of *Phyosalospora zeae*, *Macrophoma zeae*, and the gray-ear-rot fungus on four agar substrates. Columns, left to right: *P. zeae*, *M. zeae*, and the gray-ear-rot fungus. Rows, top to bottom: Malt-extract agar, potato-dextrose agar, corn-meal agar, and Czapek's-solution agar. Cultures were 40 hours old and had been grown at room temperature, 21° to 24° C.

on four media are shown in figure 2. On potato-dextrose agar these isolates grew very rapidly and a colony held at 21° to 24° C. generally covered the entire surface of the agar in a 9-cm. Petri dish within 48 hours. The mycelium of the young colony is white and of a loose, cottony texture. Within

5 to 8 days, the sub-aerial mycelium turns gray at the center of the colony; and in 14 days the entire colony, when viewed from the bottom of the plate, becomes a dark slate-gray. Later the aerial mycelium becomes progressively darker and assumes a felty texture.

Comparative growth rates of *Macrophoma zeae*, *Physalospora zeae*, and the gray-ear-rot pathogen were obtained on potato-dextrose agar. Because of the relatively rapid growth rate of these isolates, special long tubes were constructed so that measurements of the daily growth of the fungus could be made. The tubes were approximately 15 inches long and each was provided with a depression in the glass on one side near the mouth. This depression served as a dam to hold back the melted agar until it solidified. These tubes provided an agar surface about 12 inches long and $\frac{1}{2}$ inch wide on which the fungus could grow. Tubes were seeded in duplicate, each with a uniform amount of mycelium of one of the three isolates, and incubated at 21° to 24° C. Twenty-four hours were allowed for the transfers to become established and then daily measurements were made of the growth increment. The mean daily growth increment, measured for 8 days, was 32.6 mm. for *P. zeae*, 32.5 mm. for *M. zeae*, and 32.4 mm. for the gray-ear-rot fungus. This similarity in growth rate among the three isolates is further suggestive of their relationship.

In the course of studying *Physalospora zeae*, *Macrophoma zeae* and the gray-ear-rot fungus, it frequently was found that colonies ceased growth 24 to 36 hours after being transferred to fresh agar substrates in Petri plates. Sub-cultures from these colonies also failed to grow.

An experiment was set up to determine the rate of loss of viability among different cultures and factors that might influence it. Several cultures of each of the three isolates were grown in Petri plates on Czapek's-solution agar, Czapek's-solution agar plus 2 per cent autolyzed yeast, 2 per cent malt-extract agar, and potato-dextrose agar. The cultures were replicated 6 times on each medium. Transfers from each young colony were made at 24-hour intervals to fresh plates of the respective agar.

Between the 6th and 10th transfers, all cultures on those media supporting abundant and rapid growth, namely 2 per cent malt-extract agar, potato-dextrose agar, and Czapek's-solution agar plus 2 per cent autolyzed yeast, had died. The cultures carried on Czapek's-solution agar, where growth was sparse and slow, maintained their viability through 30 consecutive daily transfers, after which the experiment was discontinued. The rate of loss of viability appeared to be directly proportional to the rate of growth of the fungus. Isolates can be carried on potato-dextrose agar slants for indefinite periods, if they are not frequently transferred. It may also be pointed out that *Macrophoma zeae* and *Physalospora zeae* in host tissues lose their viability after 12 to 14 months when stored at temperatures and humidities commonly encountered in laboratories. The gray-ear-rot fungus, it has also been found, dies in ears held over 1 year in the laboratory.

EVIDENCE OF THE RELATIONSHIP OF THE GRAY-EAR-ROT FUNGUS
TO PHYSALOSPORA ZEAE

The marked similarity of *Macrophoma zeae*, *Physalospora zeae*, and the gray-ear-rot fungus in cultural behavior, growth rate, loss of viability, and the appearance of a presumably nonfunctional fruiting structure in the life cycles of all three, stimulated further efforts to determine relationship more conclusively.

Single pycnidiospore cultures of *Macrophoma zeae*, single ascospore cultures of *Physalospora zeae*, and mass transfers of the gray-ear-rot pathogen were seeded on sterilized whole wheat, polished rice, corn meal, and corn stalks. Sufficient water was added to prevent drying out. The corn stalks were placed in one-quart Mason jars, the other materials in 250-ml. Erlenmeyer flasks. All cultures were held in a greenhouse where temperatures and light intensities varied over wide ranges.

After about 6 weeks black, spherical bodies were found on the surfaces of the corn stalks. Many of these appeared to have burst, revealing masses of white pseudoparenchymatous tissue. None of the unbroken structures contained spores. At the end of 4 months (January to April) mature pycnidia were first observed. These appeared in all cultures on the sterilized corn stalks (Fig. 3, E). No fruiting bodies were observed on the other substrates. Close examination and measurement of the "second generation" pycnidiospores revealed that in size, shape, and color they were indistinguishable from the spores of *Macrophoma zeae* found on host material collected in the field. Isolates from these pycnidiospores grown in pure culture were identical in cultural behavior and growth rate with cultures of *M. zeae*, *Physalospora zeae*, and mass isolates from kernels of diseased ears.

The development, in pure culture, of pycnidiospores from isolates originating from single ascospores of *Physalospora zeae* appears to be adequate evidence that *P. zeae* and *Macrophoma zeae* are the sexual and asexual phases of the same fungus. The production of these pycnidiospores, in pure culture, from isolates derived from the gray-ear-rot fungus demonstrates rather conclusively that the heretofore unidentified ear rot is caused by *M. zeae*.

Further evidence of the identity of the ear-rot pathogen was provided by inoculation of corn ears in the field. Inoculum was prepared from young cultures of *Macrophoma zeae*, *Physalospora zeae*, and isolates from infected ears by macerating the mycelium from each and suspending the hyphal fragments in water. Inoculum was similarly prepared from cultures grown from "second generation" pycnidiospores. Each of the suspensions derived from the sources described were injected, by means of a hypodermic needle, into 30 to 60 ears of a single-cross hybrid on August 3, 1944, one week after the hybrid came into full silk. Final examination of the inoculated ears was approximately 80 days later.

Infection followed all inoculations and no differences were observable in the symptoms produced. The symptoms on artificially inoculated ears were indistinguishable from those on naturally infected ears.

THE CAUSAL FUNGUS

In 1927 Tehon and Daniels (11) adequately described *Macrophoma zeae* and the leaf symptoms produced by the fungus. The measurements and descriptions given by these authors are: Pycnidia round to oval or even lenticular in cross section, spherical to applanate in longitudinal section, developing in and occupying the mesophyll, membranous becoming carbonous with age, 65–120 μ in diameter; ostiole protruding hypophyllously, usually through a stoma, nonrostrate, oval, 28–35 \times 15–17 μ . Spores continuous, hyaline to greenish, fusiform, 17–31 \times 6.5–8.5 μ .

The measurements of material collected in Indiana (Fig. 3, B, D) are as follows: Pycnidia round to oval, 75–150 μ in diameter, found in the meso-

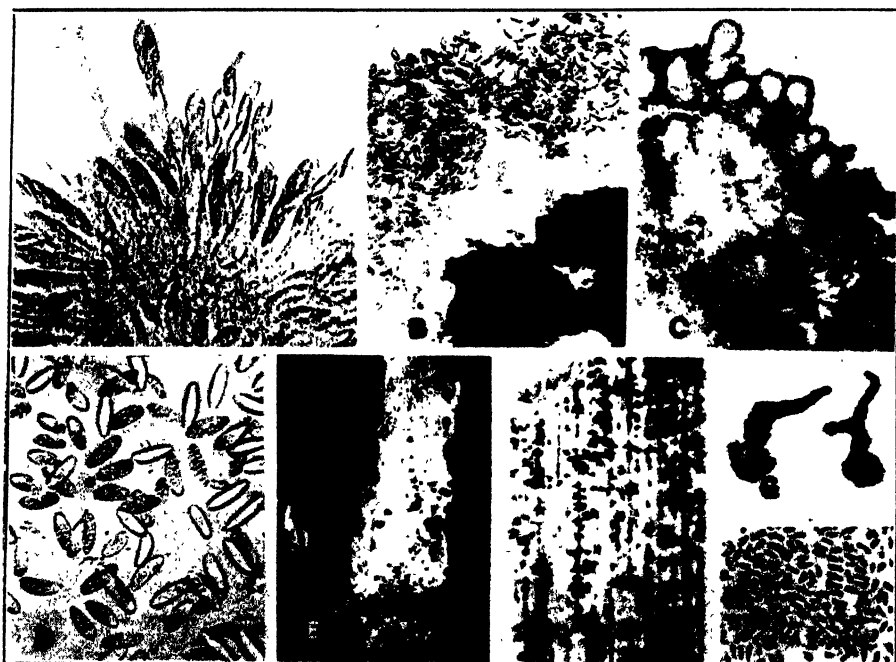


FIG. 3. Fruiting bodies and spore forms of *Physalospora zeae*. A. Asci and ascospores, $\times 150$. B. A crushed pycnidium showing pycnidiospores, $\times 60$. C. Cross section of a cluster of pycnidium-like structures produced on malt-extract agar, $\times 15$. D. Pycnidiospores, $\times 200$. E. Mature pycnidia on the surface of a corn stalk in pure culture, $\times 2$. F. Surface view of an infected tassel after 10 days in a moist chamber, showing development of pycnidium-like structures, $\times 6$. G. Pycnidium-like structure dissected out of host tissue, $\times 25$. H. Nongerminating microspores (stained), $\times 400$.

phyll, carbonous, and ostiole protruding hypophyllously. Spores 1-cell, oval to fusiform, hyaline to dilute greenish amber, 15–38 \times 7–14 μ (mean = 27.1 \times 10.2 μ). The differences noted here between the Indiana material and that of the original description are very small and may be accounted for by the normal variation expected.

Stout (10) in 1930 described *Physalospora zeae* and the symptoms it produces on corn leaves. He observed the association between *P. zeae* and

Macrophoma zeae in the same lesion and suggested that they may be perfect and imperfect stages. No cultural experiments were done to prove this connection definitely. The measurements and descriptions given by Stout for *P. zeae* are as follows:

Perithecia located in mesophyll, opening by a minutely papillate ostiole, externally carbonous, globose, 75–235 μ in diameter. Asci cylindrical, straight to curved, stalked, double-walled, outer wall thickened especially at the apex, 85–150 \times 13–22 μ . Paraphyses obscure, hyaline, filamentous. Spores 8 per ascus, arranged sub-biseriately, hyaline to very dilute olivaceous, 1-cell, narrow ellipsoid, 19–25 \times 6.5–8 μ .

The Indiana material (Fig. 3, A) had the following characters: Perithecia developed in mesophyll and protruding by a short neck, carbonous, globose, 80–250 μ in diameter. Asci cylindrical to long clavate, hyaline, double-walled, outer wall thickened at the apex, 8-spore, but occasionally 4- to 6-spore, 90–175 \times 12–18 μ . Paraphyses sparse, hyaline, filamentous, apparently branched. Spores narrow ellipsoid, tapering to narrow rounded ends, hyaline to very dilute amber, arranged obliquely in a sub-biseriate order in the ascus, 21–33 \times 8–11 μ (mean \pm 26.8 \times 10.0 μ).

The pycnidia produced in pure culture from isolates derived from single pycnidiospores of *Macrophoma zeae*, single ascospores of *Physalospora zeae*, and from mass isolates from diseased kernels differed in certain respects from those occurring on host leaves. In pure culture the pycnidia were produced in clusters on the surface of the substrate (Fig. 3, E). In nature the pycnidia are scattered and sub-epidermal, with only short necks protruding. It is interesting to note in this connection that Nishikado (8) was able to grow pycnidia of *M. reniformis* (Viala and Ravaz) Cav. in pure culture, and observed a difference in growth habit and structure from those arising in nature on infected host tissue. This author points out, however, that pycnidiospores developed in pure culture were identical with those found in nature. The "second generation" pycnidiospores of *M. zeae* grown in pure culture corresponded very closely in all respects to those occurring on diseased leaves in the field. The spores measured 14–33 \times 8.7–14 μ , with mean of 26.2 \times 10.6 μ .

The pycnidium-like structures occurring on host material in the field (Fig. 3, F, G) may be described as follows: Pycnidia occurring in mesophyll, carbonous, globose, 150–250 μ in diameter, protruding with a long neck, sometimes bifurcate and rarely trifurcate. Microspores numerous, hyaline, 1-cell, 2–4.8 \times 1–2 μ (mean \pm 4.0 \pm 1.6 μ), exuded in droplets of a hyaline mucous-like matrix, nongerminating (Fig. 3, H).

The structures occurring in pure culture on aged 2 per cent malt-extract agar and on sterilized soybean stems were slightly different (Fig. 3, C). These were found in clusters on the surface of the substrate and were without the prominent necks found in nature. The size, shape, and color of the microspores, as well as the characteristic of failing to germinate, were the same as those found on host material. The differences between these bodies

as developed in pure culture and as found on the host are of the same order as those between the functional pycnidia found in pure culture and those found on leaves in the field.

It appears reasonable to conclude that these structures are a spore form in the life cycle of *Physalospora zeae*, since they were found both in pure culture and on collections of host material. Because the spores did not germinate, continued observation on the life cycle, using these spores as a point of origin, has thus far been impossible, and the contention that they are a part of the life cycle cannot be irrefutably proved.

Preliminary observations on these apparently nonfunctional pycnidia indicate that environment may play an important part in determining their abundance. When host material was kept constantly wet in a moist chamber and under low light intensities few of these structures were produced. When material from the same lesion was placed in a moist chamber, in a greenhouse where drying out of the chamber frequently took place and light intensity was high, large numbers of these bodies were produced and functional pycnidia and perithecia developed only sparsely.

DISCUSSION

Gray ear rot has occurred sparsely in Indiana since 1939 and specimens have been collected in other sections of the Corn Belt. In 1944 the gray ear rot was widely distributed in the southern and east-central areas of the State where, in some localities, its prevalence ranged up to as high as 10 per cent.

The geographical range of the disease is unknown, but it seems likely that it will be found scattered over an appreciable portion of Eastern United States. Hoppe (5) has reported annually on the relative prevalence and geographical distribution of corn ear rots for the period from 1933 to 1942. In these reports he cited a nonsporulating unidentified fungus which occasionally reached appreciably high percentages in samples of corn collected from terminal markets on the Atlantic Seaboard. He also reported this fungus in corn samples from the Corn Belt and a few Southern States. Cultures of this fungus and of *Physalospora zeae* have been exchanged with Hoppe and have been found to be very similar in appearance and growth rate. While cultural behavior cannot be employed as positive evidence that the fungus isolated by Hoppe is *P. zeae*, yet the similarity is suggestive.

In 1933 Eddins and Voorhees (3) reported *Physalospora zeicola* Ell. and Ev., the perfect stage of *Diplodia frumenti* Ell. and Ev., as a pathogen of corn ears and stalks. This organism differs distinctly from *P. zeae* in the symptoms it produces on ears and in its morphology. The asci of *P. zeicola* measure 95-140 \times 10-13 μ , the ascospores 13-27 \times 6-11 μ . The asexual stages of these species are strikingly different from each other. The pycnidiospores of *D. frumenti* are dark, striate, and 2-cell; those of *Macrophoma zeae* are hyaline, smooth-wall, and 1-cell.

The association of the genus *Physalospora* with asexual stages in the genus *Macrophoma* has been pointed out in a number of instances. Bonar (2), by means of cultural experiment, demonstrated the connection between

P. ilicis (Schleicher) Sacc. and *M. ilicella* (Sacc. and Penz.) Berl. and Vogl. Nose (9) proved *M. kuwatsukaii* Hara to be the pycnidial stage of *P. piricola* Nose.

The rôle, if any, of the microconidia found in *Physalospora zae* is unknown. Although all attempts to germinate these spores in water or nutrient solution have been unsuccessful, it is possible that they may serve as spermatia and are able to fertilize receptive bodies, as yet unobserved, that continue their development as ascocarps. On the other hand, they may have served as a fertilizing organ which, in the course of evolution of the species, has lost its function and is unnecessary for the propagation of the fungus. It is interesting to note that Jaczewski (6) mentions the presence of microconidia ($5-5.5 \times 0.5-0.7 \mu$) in *P. baccac* Cav. and their failure to germinate. He considers these spores of doubtful significance in the spread of the fungus. Bensaude (1), on the other hand, was able to germinate the microconidia of this fungus and grow normal cultures from them. These cultures produced macroconidia similar to those encountered on the infected host.

The factors favoring spread and development of gray ear rot are not well understood at the present time. During 1944, when the disease reached the highest prevalence yet observed, weather conditions were generally dry throughout mid-summer. In those areas where the disease occurred in greatest abundance, appreciable rain fell during the latter half of August. It is possible that the summer drought favored conservation of inoculum until after silking when ears were in a receptive stage for infection. Further studies are needed to determine more precisely the factors influencing the prevalence of both the ear rot and leaf blight phases of this disease. It is unknown whether overwintering inoculum on the leaves is delayed in maturing until ears are formed or whether 2 generations of spores are produced, the second of which infects the ears. It seems reasonable that the chief source of inoculum infecting ears arises from leaf infections, either those of the previous year or of the current season. Evidence to this effect was seen in 1944 in a field in which 10 per cent or more of the ears were diseased. In November this field had an abundance of infected leaves bearing immature perithecia and pycnidia.

No control for the disease can be suggested at this time, other than the plowing under of infected corn residues. This method of control would be effective only if it were practiced by whole communities, since spread of the fungus can conceivably take place easily from field to field. If a means were known of producing large volumes of spores in pure culture, some progress might be made in differentiating resistant and susceptible inbred lines under conditions of artificially induced epidemics. Under the present circumstances, observation on the relative resistance of inbred lines and hybrids will have to be made under conditions of natural infection.

SUMMARY

The occurrence of an ear rot of corn, heretofore unidentified, is reported and its symptoms described in detail. A common name, gray ear rot, is sug-

gested, it being descriptive of the gray color of the husks and the pith of the cob.

Experiments were designed to induce the fungus associated with gray ear rot to sporulate in pure culture in order to facilitate proper identification.

In Indiana *Physalospora zeae* occurs on corn leaves and on upper internodes of the stalk below the tassel. *P. zeae* and the gray-ear-rot fungus resembled each other in cultural behavior, growth rate, and loss of viability in culture and in specimens of host material.

Cultures of the gray-ear-rot fungus and of *Physalospora zeae* grown on sterile corn stalks produced pycnidia and pycnidiospores identical with those of *Macrophoma zeae*, strongly suggesting that the gray-ear-rot pathogen is *M. zeae*, and that the latter is the asexual stage of *P. zeae*.

Further evidence of this relationship was obtained in field inoculations of corn ears, in which symptoms caused by *Physalospora zeae* were indistinguishable from those developed on ears artificially inoculated with *Macrophoma zeae* or with isolates from naturally infected ears.

A third, heretofore unrecognized, pycnidium-like fruiting structure, is believed to be a stage in the life cycle of *Physalospora zeae*.

The distribution of the disease, factors affecting its prevalence and severity, and the relationship of the fungus which causes it to other species of *Physalospora* are discussed.

No control for the disease is suggested, other than clean cultural practices.

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AND

UNITED STATES DEPARTMENT OF AGRICULTURE.

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A NEW HYPHOMYCETE PARASITIC ON A SPECIES OF NEMATODE

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In a paper published in 1941 (2), I described as new 10 nematode-destroying hyphomycetes found occurring in leaf mold and other decaying vegetable materials originating from several localities in Maryland, Virginia, and Wisconsin. Though these fungi and their biological relationships are not visible by direct examination of the opaque natural materials, they could be studied quite conveniently in transparent maize-meal-agar cultures which, after being permeated with *Pythium* mycelium, had been further planted with small quantities of the partly decomposed detritus harboring the fungi. They all operate in a typically parasitic rather than predaceous manner: their conidia become affixed externally to the host eelworms by adhesion, and then individually thrust a germ tube through the integument to invade the fleshy interior with an assimilative mycelium. The 4 species presented under the binomials *Acrotalagmus bactrosporus*, *A. oboratus*, *Cephalosporium balanoides*, and *Spicaria coccospora* closely resemble the insect parasites assigned to the same genera, except that their display of reproductive parts is far less luxuriant, owing to the much smaller size of the animals serving them as hosts. There is good reason to hold that these 4 species are intimately related to the series of entomogenous forms distributed among *Acrotalagmus*, *Cephalosporium*, *Spicaria*, and such allied genera as *Verticillium*, *Cladobotryum*, and *Beauveria*. They would seem truly kindred taxonomically to the species of *Beauveria* and *Spicaria*, for example, which because of destructiveness to the maize borer (4), the maize earworm (1), the pine bark beetle (3), and various elm insects (5), have in recent times received attention in the pages of this journal.

A fifth nematode-destroying hyphomycete of similar kinship developed abundantly in more than a dozen maize-meal-agar plate cultures that after being overgrown with mycelium of *Pythium ultimum* Trow had been further planted with small quantities of partly decayed crabgrass (*Digitaria sanguinalis* (L.) Scop.) refuse collected from several weed piles near a community vegetable garden in Arlington, Virginia, on April 25, 1944. Although several species of nematodes present in the decaying refuse multiplied freely in the cultures, the fungus apparently restricted its attack to eelworms belonging to a single species, which Dr. G. Steiner has kindly identified as *Panagrolaimus subclongatus* (Cobb) Thorne. Growth of the parasite within infected specimens could not be followed successfully while invasion was progressing, as all fleshy host structures, except the conspicuously resistant oesophagus and oesophageal bulb, soon undergo globuliferous degeneration

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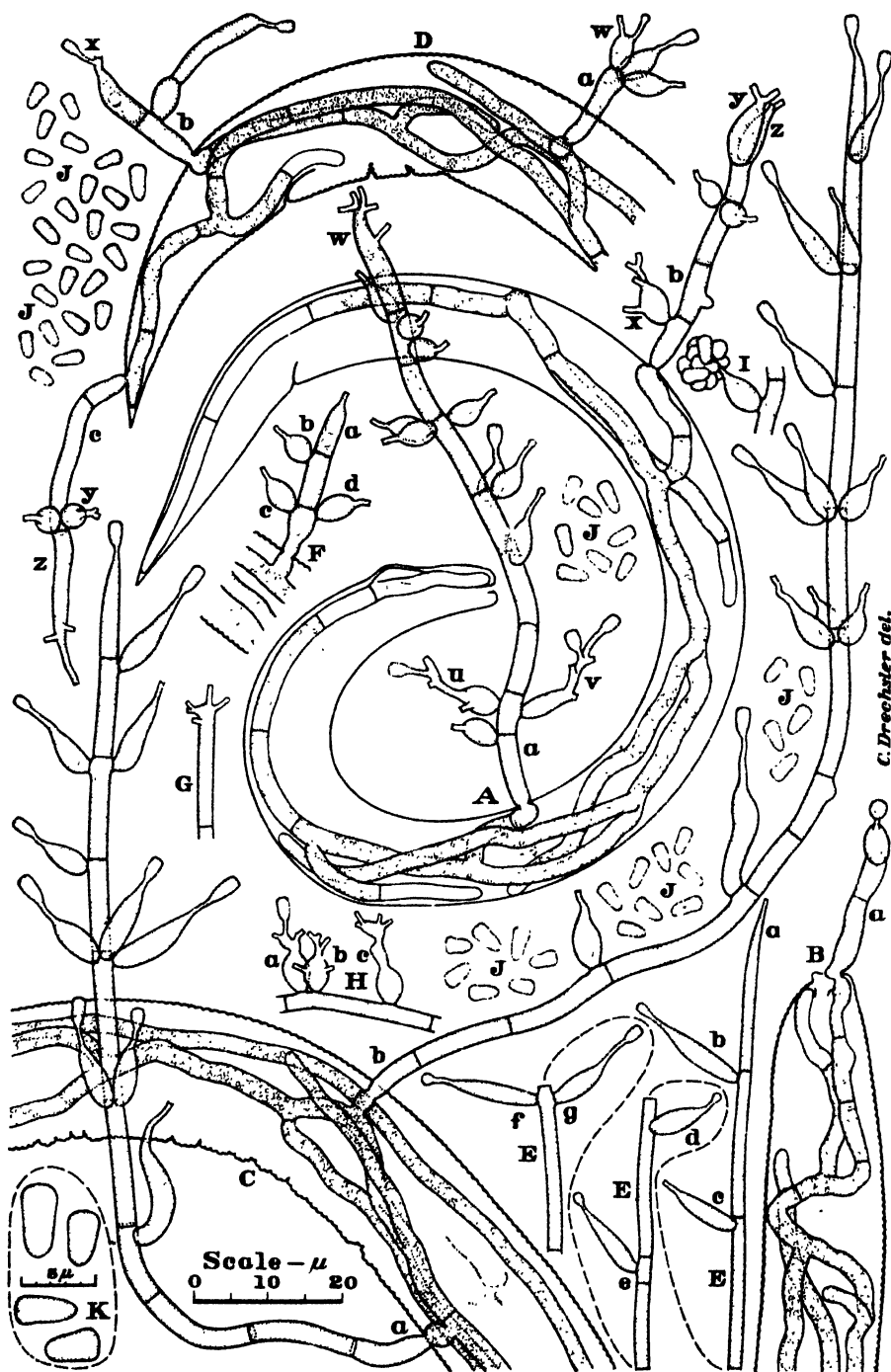


FIG. 1. *Acrostalagmus zcosporus*, drawn to a uniform scale with the aid of a camera lucida. Magnification: A-J, $\times 1000$; K, $\times 2000$.

and thereby offer optical difficulties that prove all the more troublesome since the contents of the young hyphae are likewise pronouncedly globuliferous. After the host contents, apart from the rather slowly evanescent oesophagus and bulb, have been largely assimilated by the fungus, and have been utilized for the production externally of conidiophorous filaments (Fig. 1, A, a, b; B, a; C, a, b; D, a-c), the assimilative mycelium is more clearly discernible. In small eelworms it may consist only of a single axial filament extending from head to tail, together with half a dozen branches of variable lengths (Fig. 1, A). Animals of greater dimensions show correspondingly more profuse hyphal development (Fig. 1, B, C), though in these also the tapering tail is usually occupied by only a single filament (Fig. 1, D). As long as the hyphae remain densely filled with globulose contents few cross-walls can be seen in them (Fig. 1, C). It is uncertain whether at this stage cross-walls are actually few or whether they are in large part obscured owing to the nature of the protoplasm wherein they are immersed. At all events, after the hyphal contents have suffered some reduction in density from the continuous transfer of substance required for formation of conidial apparatus, partitions may be distinguished at fairly close intervals (Fig. 1, A, B). Before long, further transfer of substance entails complete withdrawal of contents from terminal segments of the mycelium (Fig. 1, A, D), and thereupon evacuation of other segments proceeds until the empty host integument surrounds only the equally empty and rapidly evanescent envelopes of the assimilative filaments.

As in allied forms the character of the conidial apparatus produced by the fungus is greatly influenced by positional relationships to the substratum. In instances where the animal host has succumbed on the surface of the substratum the conidiophorous hyphae may at once grow erectly or ascendingly into the air (Fig. 1, A, a, b; D, a; F); or they may grow procumbently for some distance, and then continue their further growth ascendingly (Fig. 1, C, a, b); or, again, they may conclude their growth while still procumbent throughout their length (Fig. 1, B, a; D, b, c). In instances where the animal host has succumbed under the substratum, but not in a deeply submerged position, the conidiophorous hyphae usually make their way to the surface, often by widely divergent paths, before giving rise to conidiiferous branches, or phialides, from their prostrate or their ascending prolongations. Where, however, the animal has succumbed deep under the substratum the conidiophorous hyphae mostly fail to reach the surface, and will then give rise to phialides in submerged positions. Submerged and prostrate portions of conidiophorous hyphae show generally a rather widely spaced arrangement of phialides; many of the segments here being sterile, many others bearing only a single phialide (Fig. 1, B, a; C, b; E, a-e), and few bearing as many as 2 phialides (Fig. 1, E, f, g). In the ascending portions of conidiophorous hyphae, on the other hand, virtually all segments are fertile, with many of them bearing 2 or 3 phialides.

For the most part the phialides are of the familiar flask-shape type that

taper off distally into a single sterigma (Fig. 1, C, a, b; E, a-g). However, instances in which they support plural sterigmata are not altogether infrequent (Fig. 1, A, u-z; D, w-z; G; H, a-c). In such instances 2 (Fig. 1, D, w), 3 (Fig. 1, D, z), or 4 (Fig. 1, G) sterigmata often project separately from the same cell, and must have been put forth independently of one another; but often, again, a rachiform arrangement of sterigmatic tips gives evidence that an apical sterigma, after abstricting some conidia, grew out below its apex to form a second sporogenous tip (Fig. 1, D, x, y), which in turn gave rise to a third (Fig. 1, H, a) and possibly, on repetition of the process, to a fourth and a fifth sporogenous tip (Fig. 1, A, v). Frequently where plural sterigmata of separate origin are present, some may remain simple while one or more of the others undergo successive *Beauveria*-like prolongation (Fig. 1, A, u, w, x; G; H, b); so that in the end the phialide may offer a rather promiscuously appendaged aspect suggestive of the phialides figured by Petch (7) for his *Cladobotryum ovalisporum*.

It may be presumed that phialides with multiple sterigmata are generally somewhat more prolific than those of unitary make-up. The latter, as a rule, abstrict 5 to 15 conidia, which remain attached in a cohering cluster (Fig. 1, J). Clusters formed aloft in the air disintegrate when the structures supporting them finally collapse; the spores then are left strewn about on the substratum, ready to adhere apically to any specimen of the host nematode that may visit the seeded area. They recall rather strongly the conidia of *Cephalosporium balanoides*, being similarly flattened at the broad distal end and rounded at the narrower proximal end, but owing to their greater length, they show in longitudinal profile (Fig. 1, J, K) less resemblance to an acorn than to a maize (*Zea mays* L.; kernel. A term compounded in part of the generic name of maize is accordingly adopted as an appropriate epithet for the fungus. In view of its frequently liberal production of ascending conidiophorous hyphae, the species is assigned to *Acrostalagmus*, though its varied expression of reproductive habit might be more fully taken into account by resorting to the double citation "*Cephalosporium (Acrostalagmus)*" preferred by Petch (6) in naming related entomogenous forms.

Acrostalagmus zeosporus sp. nov.

Mycelium nutritum incoloratum, ramosum, septatum, intra vermiculos nematoideos viventes evolutum, in hyphis 2-3 μ crassis constans. Hyphae fertiles extra animal mortuum evolutae, subinde in materia animal ambiente omnino immersae sed saepius magna parte repentes vel ascendentes, hyalinae, vulgo simplices sed subinde ramosae, 10-300 μ longae, plerumque 1.8-3 μ crassae, in cellulis plerumque 5-25 μ longis constantes, plerisque cellulis 1-3 ramulos conidiferos sursum ferentes; ramulis conidiferis (phialis) vulgo lageniformibus, 5-20 μ longis, 2.5-4 μ crassis, plerumque sursum in unico sterigmatate 0.6-0.8 μ crasso abeuntibus et 5-15 conidia gignentibus, quandoque tamen 2-4 sterigmata ex ventre proferentibus; sterigmatibus quandoque prope apicem deinceps repullulantibus ad modum *Beauveriae*; conidiis primo cohaerentibus, continuis, incoloratis, inversum elongato-nuciformibus, apice aliquid complanatis, deorsum attenuatis, basi rotundatis, plerumque 3.5-4.6 μ longis, 1.7-2.1 μ crassis.

Panagrolaimum subelongatum necans habitat in foliis caulibusque *Digitalis sanguinalis* putrescentibus in Arlington, Virginia.

Assimilative mycelium colorless, branched at moderate intervals, septate, growing within living nematodes, composed of filamentous hyphae 2 to 3 μ wide. Conidiophorous

hyphae developed outside of dead animal host, sometimes immersed entirely in the surrounding material, but more often largely prostrate or ascending, colorless, commonly unbranched but occasionally branched, 10 to 300 μ long, often 1.8 to 3 μ wide, consisting of cells mostly 5 to 25 μ long, the terminal one, often 25 μ long and as little as 1.5 μ wide, abstricting conidia at its narrow apex, the others mostly bearing 1 to 3 conidiiferous branches (phialides) at the forward end; conidiiferous branches (phialides) commonly flask shaped, 5 to 20 μ long, 2.5 to 4 μ wide, mostly abstricting 5 to 15 conidia from a single terminal sterigma, but occasionally putting forth 2 to 4 separate sterigmata; the sterigmata whether present singly or plurally sometimes through successive subapical elongation forming one or more additional sporogenous tips in zigzag arrangement; conidia first cohering in a cluster, continuous, colorless, inversely elongate-nuciform, somewhat flattened at the distal end, narrowing downward, rounded at the proximal end, mostly 3.5 to 4.6 μ long and 1.7 to 2.1 μ wide.

Parasitizing *Panagrolaimus subelongatus* it occurs in decaying leaves and stems of *Digitaria sanguinalis* in Arlington, Virginia.

In connection with the morphological tendencies evident in the new species it may be appropriate to mention here that more than half a century ago a mold very similar to *Botrytis* (now *Beauveria*) *bassiana* was briefly noted by Zopf (8, p. 340) among several fungi he had observed causing nematode diseases.

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THE EFFECTS OF SOIL MOISTURE AND TEMPERATURE ON FUSARIUM WILT OF TOMATO

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During a ten-year study of the Fusarium wilt disease of tomato in connection with a breeding program for the production of wilt-resistant varieties, and the testing of wilt resistance in various commercial varieties, the author observed that John Baer, a susceptible variety, and Marglobe, a resistant variety, seemed to differ in their responses to climatological conditions as related to susceptibility to this disease.

PRELIMINARY RESULTS

The method employed in these wilt-resistance tests has been described in detail elsewhere (13). John Baer and Marglobe were tested in the wilt-infested field each season for comparison with the selections. The same seed stock of these commercial varieties was used each year. When the wilt records of these two varieties for the ten-year period were compared with seasonal temperature and rainfall records of the U. S. Weather Bureau, a very definite relationship between incidence of wilt and soil moisture was indicated that could hardly be coincidental. The graph in figure 1 shows the comparison between the weather data and the wilt records obtained in the field tests.

With the exception of 1933, there was more wilt infection in John Baer in seasons of greater rainfall and less wilt infection when there was less rainfall. However, just the reverse effect seems to occur with Marglobe, *i.e.*, there was a greater incidence of wilt in seasons of less rainfall and less wilt in seasons of greater rainfall. There appeared to be no particular correlation between air temperature and wilt.

REVIEW OF LITERATURE

This preliminary observation is not wholly in accord with that of Humbert (7) and others who state that Fusarium wilt of tomato is favored by dry, hot weather. In an effort to determine whether or not the apparently opposite responses in wilt susceptibility of these two varieties to rainfall as indicated in figure 1 were actual, some tests at constant soil temperature and moisture levels were undertaken.

The relationships between soil temperature and tomato wilt and between temperature and the growth of the causal organism have been well investigated, but little attention has been devoted to a study of the soil moisture relations of this disease. As early as 1920, Edgerton and Moreland (3) determined the optimum temperature for the growth of this fungus. In

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1927, White (18) reported the optimum growth temperature of many different isolants to range from 24° to 28° C., and in 1928, Haymaker (6) stated that a soil temperature of 28° C. was optimum for both the growth of the fungus and the development of the disease. Clayton (1) found that the optimum soil temperature for the development of wilt was 28° C. and the optimum air temperature was from 27° to 35° C.

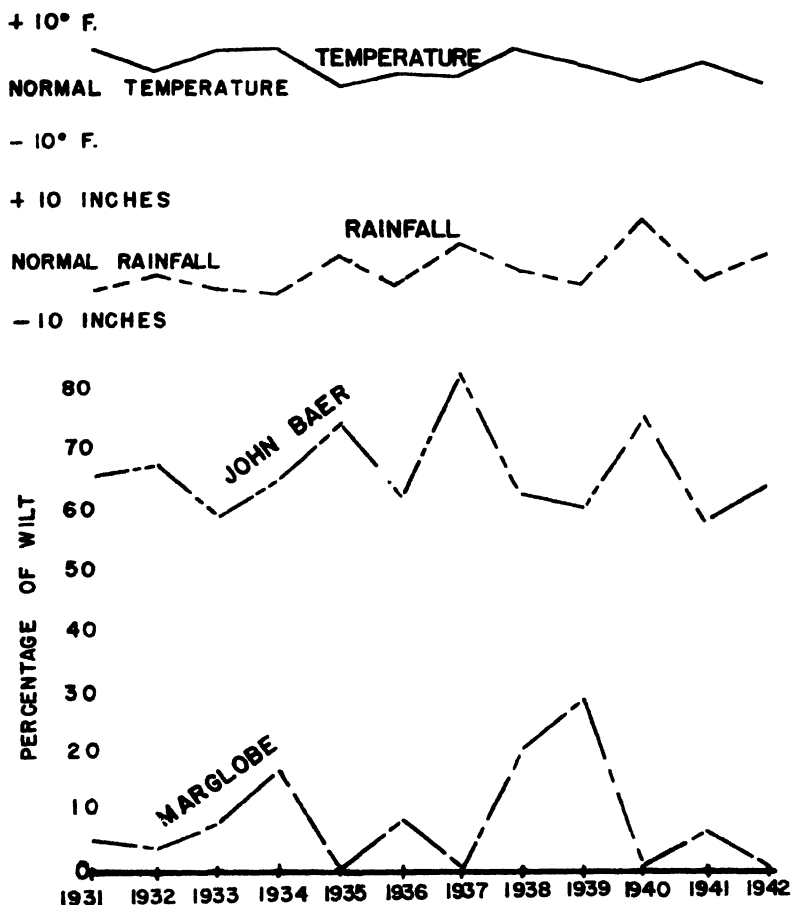


FIG. 1. Graphs showing yearly incidence of Fusarium wilt in two varieties of tomato compared with temperature and rainfall curves for the corresponding seasons.

Clayton (2) also investigated the relation of soil moisture to Fusarium wilt of tomato and reported that plants growing in low-moisture soils were resistant. Plants growing in soil of low moisture content lost their disease resistance if a rapid vegetative growth was induced by the addition of moisture to the soil. Rapidly growing plants which were susceptible were made resistant by allowing the soil to dry out.

Others have studied the effects of soil temperature and moisture on closely related diseases of other crops. Jones and Tisdale (10) stated that

the optimum soil temperature for the development of flax wilt was the same as the optimum temperature for growth of the fungus. Walker (17) reported on the relation of soil temperature to watermelon wilt. Tisdale (16) found that a susceptible variety of cabbage was more susceptible to yellows at a low moisture level than at a higher moisture level. Tims (15) tested both susceptible and resistant varieties of cabbage and found the percentage of infection of both increased with increasing soil temperatures from 20° to 32° C. Only one soil moisture experiment was conducted and "the results were too variable to be of much value."

Elliot (4) stated that cotton wilt was more severe in wet seasons, while Neal (11) held that it was more serious in dry, hot seasons. Neal (12) and Young (19) agreed that the soil temperature most favorable to the growth of the causal fungus was also most favorable for the development of cotton wilt. Tharp and Young (14) found that a susceptible variety of cotton showed positive correlation of disease with a rise in moisture level from 20 to 90 per cent of saturation, while a resistant variety showed little correlation between disease and moisture level.

TESTS WITH CONTROLLED SOIL TEMPERATURE AND MOISTURE CONDITIONS

The constant soil temperature tanks employed in the tests reported here were of the Wisconsin type described by Jones (7, 8) with a heater and thermostat in each tank. As previous investigators had found 28° C. the optimum for the development of *Fusarium* wilt, this temperature and a lower one, 22° C., were selected for these tests. Two moisture levels, 60 and 85 per cent of saturation of the soil, were also tested. The varieties used were Marglobe and John Baer from the same seed stocks as were employed in the field tests.

In the first tests in 1939 the seeds were planted in sterile soil and the three-weeks-old seedlings were transplanted into the cans. A loam, previously sterilized, was used in the cans, and its moisture content was adjusted to the desired levels. Oat cultures of the causal fungus were added to the soil to furnish inoculum. After the plants were set, a 1-inch layer of ground cork was added on top of the soil to retard radiation and evaporation. The cans were weighed daily and moisture levels maintained by adding distilled water daily or as necessary.

Five plants were in each can, and twenty plants in each test. Five non-inoculated plants of each variety were grown at all soil moisture and temperature levels for comparison as to size.

An air temperature of approximately 22° C. was satisfactorily controlled by thermostat except on bright days when regulation of the ventilators was necessary.

In the first series of tests, in 1939, the development of wilt was observed for 10 weeks. When the final readings were made, the plants were pulled and the basal portions of the stems were cut across and lengthwise so that

browning of the vascular bundles might be observed. This examination was used to verify external symptoms. The final record included dead plants as well as those in various stages of disease. The results of these tests are presented in table 1.

The susceptible variety, John Baer, was more susceptible to wilt at the higher moisture level while the resistant variety, Marglobe, was more susceptible at the lower moisture level. As to the effect of soil temperature, these data show that (with one exception) there was a greater incidence of wilt in both varieties at the higher temperature. A comparison of the non-inoculated control plants showed that both varieties produced larger plants at the higher soil moisture and temperature levels. None of the noninoculated plants developed the disease.

TABLE 1.—*The effect of two constant soil moisture and temperature levels upon the development of Fusarium wilt in a resistant and a susceptible variety of tomato. (Twenty plants in each test)*

Variety	Soil moisture content, per cent	Soil temp.	Percentage of inoculated plants infected ^a
Marglobe (resistant)	85	22° C.	20
	85	28	30
	60	22	40
	60	28	40
John Baer (susceptible)	85	22	60
	85	28	90
	60	22	50
	60	28	70

^a None of the noninoculated control plants developed wilt.

In the winter of 1939-40, these tests were repeated with some innovations. The same numbers of plants were grown in infested soil at each moisture and temperature level for the first three weeks of the experiment, and then half of them were allowed to continue for the remainder of the period with conditions unchanged while the other half were subjected to changes in soil temperature or moisture for the remaining three weeks of the experiment. Twice as many noninoculated control plants were grown as in the previous series and half of these were subjected to the same changes as the inoculated plants.

On Jan. 11, 1940, the basal portions of the stems were examined for discoloration of the vascular system. The percentages of diseased plants occurring under the various sets of conditions were recorded, and are summarized in table 2.

None of the noninoculated plants developed wilt. Changes in soil moisture and temperature levels during the experiment made but slight differences in the final size of the noninoculated plants. Plants of both varieties were larger at the higher soil moisture and temperature levels.

The results obtained in the previous tests were corroborated. Marglobe had more wilt infection at the lower moisture level, while more wilt developed

TABLE 2.—*The effects of constant and changing soil moisture and temperature levels on the development of Fusarium wilt in a resistant and a susceptible variety of tomato during a six-weeks period. Temperature and moisture changes were made at the end of the first three weeks. (Ten plants in each test)*

Variety	Soil moisture content, per cent	Soil temp.	Condi- tions constant	Percentage of inoculated plants infected ^a			
				Moisture constant		Temp. constant	
				Temp. changes		Moisture changes	
Marglobe (resistant)	85	22° C.	20			85 → 60	40
	85	28	20	28° → 22°	20		
	60	22	30	22° → 28°	40		
	60	28	50			60 → 85	40
John Baer (susceptible)	85	22	80			85 → 60	60
	85	28	90	28° → 22°	90		
	60	22	60	22° → 28°	80		
	60	28	70			60 → 85	90

^a None of the noninoculated control plants developed wilt.

in John Baer at the higher moisture level. At a constant soil temperature, an increase in soil moisture from 60 to 85 per cent of saturation, decreased the percentage of wilt in Marglobe and increased it in John Baer: the opposite change from 85 to 60 per cent saturation increased the percentage of wilt in Marglobe and decreased the percentage of wilt in John Baer.

In the winter of 1941-42, these tests were repeated using the same seed stock and method except that the inoculation was accomplished by dipping the roots of the seedlings in a suspension of the fungus, as described by Harrison (4), at the time of transplanting into the cans. The noninoculated controls were dipped in sterile water. In this series, a larger number of inoculated plants was used, so that all the possible changes in soil tempera-

TABLE 3.—*A comparison of the effects of constant and changing soil moisture and temperature levels on the development of Fusarium wilt in a resistant and susceptible variety of tomato during a six-weeks period (Dec. 3, 1941, to Jan. 12, 1942). Temperature and moisture changes were made at the end of the first three weeks. (Ten plants in each test)*

Variety	Soil moisture content, per cent	Soil temp.	Condi- tions constant	Percentage of inoculated plants infected ^a			
				Moisture constant		Temp. constant	
				Temp. changes		Moisture changes	
Marglobe (resistant)	85	22° C.	20	22° → 28°	40	85 → 60	40
	85	28	40	28 → 22	40	85 → 60	60
	60	22	40	22 → 28	50	60 → 85	30
	60	28	60	28 → 22	60	60 → 85	40
John Baer (susceptible)	85	22	90	22 → 28	100	85 → 60	80
	85	28	100	28 → 22	100	85 → 60	90
	60	22	70	22 → 28	80	60 → 85	100
	60	28	80	28 → 22	80	60 → 85	100

^a None of the noninoculated control plants developed wilt.

ture and moisture could be made and the effects on wilt compared with inoculated plants grown at constant soil moisture and temperature levels.

The tests were set up in the tanks on Dec. 3, 1941, the changes in growing conditions made Dec. 24, and the percentages of wilt were recorded Jan. 12, 1942, when the basal portions of the stems were examined for discoloration of the vascular system. The results of this series of tests are given in table 3.

These data are in accord with those obtained in the two previous tests. Under constant conditions, more wilt developed in Marglobe at the lower moisture level than at the higher level, while more wilt developed in John

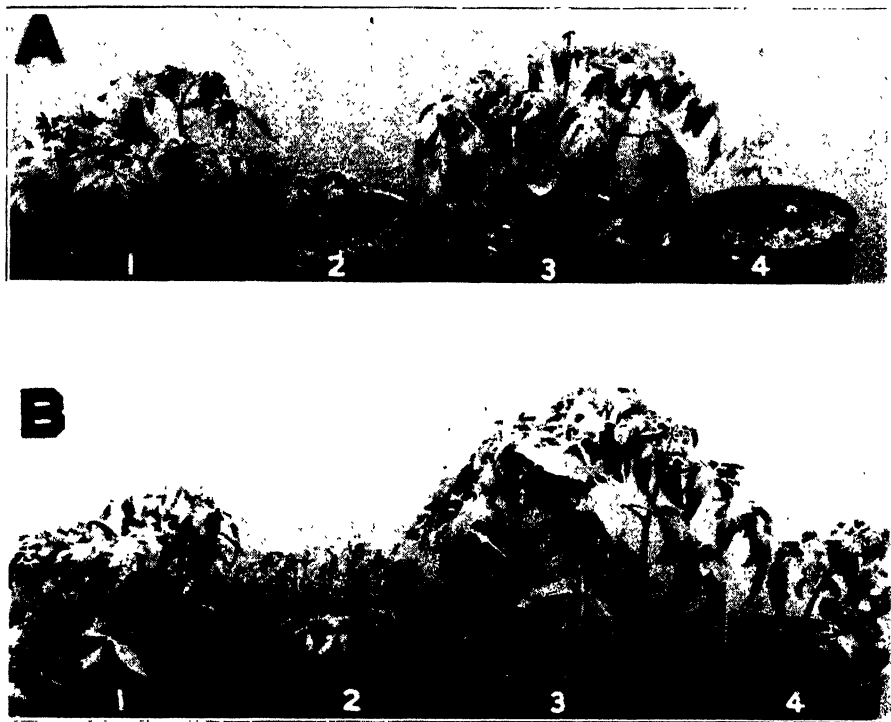


FIG. 2 John Baer (A) and Marglobe (B) tomato plants grown at 28° C. Non-inoculated control (1) and inoculated plants (2) at 60 per cent moisture; noninoculated control (3) and inoculated plants (4) at 85 per cent moisture.

Baer at the higher moisture level than at the lower one. In both varieties, the incidence of wilt was greater at the higher soil temperature level. Under changing conditions, at both temperature levels an increase in soil moisture decreased the percentage of wilt in Marglobe, while a decrease in soil moisture increased the percentage of wilt. In the case of John Baer just the reverse was true. At constant moisture levels, the incidence of wilt increased in both varieties with a rise in temperature from 22° to 28° C. while a decrease in temperature from 28° to 22° C. showed no effect on the incidence of this disease.

Photographs of some of the plants of the final experiments are in figure 2.

These pictures compare noninoculated control plants with inoculated plants grown at the same soil moisture and temperature levels. The noninoculated plants of both varieties made better growth at the higher moisture and temperature levels.

DISCUSSION

The results of the three series of tests under controlled conditions of soil moisture and temperature substantiate the apparent relationship between wilt incidence and rainfall shown in figure 1.

These findings corroborate the general observation that hot weather favors wilt, but indicates that dry weather does not favor wilt in all cases. The difference between varieties in response to soil moisture may account for the variation in reports as to the effects of wet and dry seasons on this disease.

The results reported here with John Baer are in accord with those of Clayton (1, 2) who used Chalk's Early Jewel in his investigations. Chalk's Early Jewel is closely related to John Baer, and is susceptible to wilt.

The findings with a susceptible variety of tomato recorded here are in agreement with those of Tharp and Young (13) who found that a susceptible variety of cotton showed a positive correlation of disease with a rise in moisture level from 20 to 80 and 90 per cent saturation. These investigators used a resistant variety in only one test and reported little correlation between disease and soil moisture level.

The effects of soil temperature on the development of wilt reported here are in accord with those of other investigators (1, 6, 10, 14, 15, 16, 18).

Whether the difference in effect of soil moisture on wilt incidence is correlated with susceptibility or resistance to the disease, or is merely a varietal difference has not been established in these trials as only one susceptible and one resistant variety were tested under controlled conditions. Since field observations on other varieties were sometimes based on only two trials which were often not in subsequent years, no definite conclusions as to moisture relations can be made. Pritchard, a resistant variety which was tested in the infested field for four years, showed the same response in wilt incidence to rainfall as Marglobe, while the wilt resistant strain of John Baer (13) responded in the same manner as commercial John Baer.

SUMMARY AND CONCLUSIONS

Field observations on the relation of rainfall to the incidence of *Fusarium* wilt and greenhouse tests at constant soil moisture and temperature levels indicate that Marglobe, a resistant variety, and John Baer, a susceptible variety of tomato, have opposite responses to soil moisture insofar as it affects susceptibility to wilt.

Marglobe was more susceptible to wilt at a soil moisture level of 60 per cent saturation than at 85 per cent, while John Baer was more susceptible to wilt at a soil moisture level of 85 per cent saturation than at 60 per cent.

When soil temperature conditions were constant and soil moisture conditions were changed during the tests, a reduction in soil moisture decreased the incidence of wilt in John Baer, while an increase in soil moisture increased the incidence of wilt. Conversely, in Marglobe an increase in soil moisture from 60 to 85 per cent saturation reduced the incidence of wilt, while a reduction in moisture increased the incidence of wilt.

The responses in wilt susceptibility of John Baer and Marglobe to soil temperatures are similar. Both varieties were more susceptible to wilt at 28° C. than at 22° C. When soil moisture conditions were constant and soil temperatures were changed during the tests, a rise from 22° to 28° C. usually increased the incidence of wilt in both varieties, but a reduction in soil temperature from 28° to 22° C. had no effect on the percentage of wilt which developed.

Whether the difference in effect of soil moisture on wilt incidence is correlated with susceptibility or resistance to the disease, or is merely a varietal difference has not been established in these trials as only one susceptible and one resistant variety were tested.

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INHERITANCE OF REACTION TO CROWN RUST AND STEM RUST AND OTHER CHARACTERS IN CROSSES BETWEEN BOND, AVENA BYZANTINA, AND VARIETIES OF *A. SATIVA*¹

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INTRODUCTION

Plant diseases are important limiting factors in the production of oats. Crown rust caused by the pathogen *Puccinia coronata avenae* Erikss. and stem rust caused by *P. graminis avenae* Erikss. and Henn. are two of the more destructive diseases of oats. The modes of inheritance of crown-rust and stem-rust reaction and of several kernel characters are reported herein for crosses between Bond, *Avena byzantina* C. Koch, and several varieties of *A. sativa* L.

The literature concerning the inheritance of crown-rust and stem-rust reaction in oats has been reviewed by Smith (5) and Torrie (7). Hayes, Moore, and Stakman (1) studied crown-rust reaction in crosses involving Bond and 5 different varieties of *Avena sativa*. In 1 of the 5 crosses they found a single factor difference, while in the other 4, two incompletely dominant complementary factors for resistance were carried by Bond. Torrie (7) explained crown-rust reaction in the cross of Iowa No. 444 × Bond on the basis of one factor pair for resistance carried by Bond and an inhibitor carried by Iowa No. 444 which partly inhibited the expression of the factor for resistance. Previous studies (5, 7) have shown that resistance to stem rust is dominant to susceptibility and dependent upon a single factor pair.

The literature up to 1939 on the inheritance of kernel characters has been reviewed by Torrie (7). Hayes, Moore, and Stakman (1) found that the *Avena sativa* type of basal articulation was dominant over the *A. byzantina* type, the ratio approaching 3 : 1. They reported that segregation for floret disjunction (termed rachilla attachment in this paper) in 1 of the 5 crosses studied was monogenic, while in the other 4 crosses it was dependent upon more than a single factor pair for differentiation. In a cross of Iowa No. 444 × Bond, Torrie (7) reported that the characters basal hair length and

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number and basal articulation and rachilla attachment were monogenic in their inheritance, while lemma color and awnness were digenic.

The literature on the inheritance of earliness of heading has been reviewed recently by Torrie (7). He reports that other workers found a partial dominance of earliness and that one to multiple factors, depending upon the cross, are suggested to explain the segregation.

MATERIALS AND METHODS

The progenies used in these studies consisted of the following:

F_2 , F_3 , and F_4 of Bond (C.I.³ 2733) \times Hawkeye (C.I. 2464)

F_2 and F_3 of Bond \times S.D. 334 (C.I. 2884)

F_2 and F_3 of Hawkeye \times Bond

F_2 and F_3 of S.D. 334 \times Bond

F_2 and F_3 of Hancock (C.I. 3346) \times Bond

F_2 and F_3 of C.I. 3350 \times Bond

These varieties belong to the species *Avena sativa*, with the exception of Bond, which belongs to *A. byzantina*. The origins of the parental varieties, with the exception of S.D. 334 and C.I. 3350 have been reported by Stanton (6). S.D. 334 is a selection made at the South Dakota Agricultural Experiment Station from a selection of Swedish Select \times Kilby Hulless back-crossed twice to Richland (C.I. 787) and then to Markton (C.I. 2053). C.I. 3350 is a sister selection of Hancock and Marion.

The F_2 and F_3 generations of Bond \times Hawkeye were grown successively in 1938 and 1939. The F_2 generation of Bond \times S.D. 334 was grown in 1937 and the F_3 generation both in 1938 and 1939, and the F_2 of the remaining crosses in 1939 at the University Hill Farms, Madison. The F_3 lines were planted in 5-foot rows with approximately 25 seeds per row. Parental varieties were seeded at intervals of 30 rows. Border rows of State Pride (C.I. 1154) were planted throughout the nursery to provide a susceptible host for the increase of crown and stem rusts. During 1937 and 1938 naturally occurring inoculum was relied upon for rust infection. In 1939 the natural inoculum was supplemented by inoculating growing plants of State Pride by means of a hypodermic needle.

The seedling reactions to a composite collection of crown rust were determined in the greenhouse. The F_3 generations of Bond \times Hawkeye and Bond \times S.D. 334 were grown during the winter of 1938-39 while the F_4 of Bond \times Hawkeye and the F_2 and F_3 progenies of the other cross were studied during the winter of 1939-40. The crown-rust reactions of 94 F_3 lines of Bond \times Hawkeye, selected at random, were determined for physiologic races 3 and 6 and for the composite inoculum during the winter of 1939-40. The seedlings were grown in flats. The first leaf of the seedling plant was inoculated with crown rust. The flats containing the inoculated plants were placed in a lighted moist-chamber for 24 hours at 20° C., after which they were removed to a greenhouse kept at approximately 18° C.

³ C.I. refers to accession number of the Division of Cereal Crops and Diseases.

All plants, both in the adult plant and seedling stages, were classified for crown-rust reaction into 5 classes: 0, 1, 2, 3, and 4 based on the infection types described by Murphy (4). A large majority of the plants was placed in classes 0, 1, and 4. In the genetic analysis of the data, classes 0, 1, and 2 were grouped as resistant and 3 and 4 as susceptible. All plants were classified for stem-rust reaction as either resistant or susceptible.

The classification for grain characteristics was made in the laboratory by examination of 3 to 4 caryopses of each plant by 2 persons. Three classes were used for all characters: byzantina, sativa, or intermediate type.

The dates when the first, approximately 50 per cent, and the last plant headed were determined for each F₁ and parental line of the crosses Bond × Hawkeye and Bond × S.D. 334. The arithmetic mean of the above three values was taken as an index of the number of days from emergence to heading.

EXPERIMENTAL RESULTS

Inheritance of Crown-Rust Reaction

F₁ plants of all crosses studied had a 1 to 2 crown-rust infection type in the seedling stage under greenhouse conditions and a 3 type in the adult plant stage in the field where the composite inoculum was used.

Data on the F₂ segregation are given in table 1. Good fits were obtained to the ratio of 9 resistant : 7 susceptible in the seedling stage and 5 resis-

TABLE 1.—Crown-rust reactions of the F₂ progenies of Bond (*Avena byzantina*) crossed with several varieties of *A. sativa*, and tests of goodness of fit to the 5 resistant : 11 susceptible and 9 resistant : 7 susceptible ratios in the adult and seedling stages, respectively

Cross and population	Stage of development	Reaction classes		χ^2 values ^a
		Resistant	Susceptible	
		No. of plants	No. of plants	
Bond × Hawkeye	Adult	82	171	0.10
Hawkeye × Bond	Adult	34	91	1.10
	Seedling	164	156	3.05
Bond × S.D. 334	Adult	16	176	1.08 ^b
S.D. 334 × Bond (2974)	Adult	41	74	0.82
	Seedling	104	121	8.79
S.D. 334 × Bond (2975)	Adult	25	90	4.46
	Seedling	139	131	4.39
S.D. 334 × Bond (2976)	Adult	44	95	0.00
	Seedling	163	135	0.23
Hancock × Bond (2988)	Adult	31	74	0.97
	Seedling	97	71	0.10
Hancock × Bond (2989)	Adult	36	68	2.96
	Seedling	89	66	0.05
C.I. 3350 × Bond (2983)	Adult	43	71	1.90
	Seedling	112	100	0.87
C.I. 3350 × Bond (2984)	Adult	32	92	1.34
	Seedling	80	64	0.01

^a The χ^2 values at the 5 per cent and 1 per cent levels for 1 degree of freedom are 3.84 and 6.64, respectively.

^b χ^2 values for the ratio of 15 susceptible: 1 resistant.

tant: 11 susceptible in the adult plant stage save for the seedling reactions of populations 2974 and 2975 of S.D. 334 × Bond, and the adult plant reaction of population 2975. In the latter 3 instances the proportion of plants classified as susceptible exceeded that expected. The adult plant segregation of Bond × S.D. 334 approached the ratio of 15 susceptible to 1 resistant.

The crown-rust reactions of the F_2 generations of the several crosses and for a selected F_4 of Bond × Hawkeye are given in table 2. The F_4 generation

TABLE 2.—Crown-rust reactions of F_2 and F_4 progenies of Bond (*Avena byzantina*) crossed with several varieties of *A. sativa*, and tests of goodness of fit to the 1 resistant: 4 segregating (3 resistant: 1 susceptible): 4 segregating (9 resistant: 7 susceptible in the seedling stage and 5 resistant: 11 susceptible in the adult stage): 7 susceptible ratio

Generation, cross, and population	Indicated reaction in seedling stage				χ^2 values ^b
	Resistant	Segregating		Susceptible	
		3R : 1S ^a	9R : 7S		
F ₂ Bond × Hawkeye	19	73	57	104	3.17
F ₄ Bond × Hawkeye ^c	6	58	59	97	4.83
F ₂ Hawkeye × Bond	6	24	32	47	1.33
F ₂ Bond × S.D. 334	9	12	21	149	1.30 ^d
F ₂ S.D. 334 × Bond (2974)	10	22	18	60	8.71
F ₂ S.D. 334 × Bond (2975)	6	18	27	55	4.38
F ₂ S.D. 334 × Bond (2976)	10	27	28	45	1.61
F ₂ Hancock × Bond (2988)	12	27	25	40	5.40
F ₂ Hancock × Bond (2989)	8	24	24	48	0.79
F ₂ C.T. 3350 × Bond (2983)	9	28	30	42	1.73
F ₄ C.T. 3350 × Bond (2984)	7	30	30	49	0.14

Generation and cross	Indicated reaction in adult stage				χ^2 values
	Resistant	Segregating		Susceptible	
		3R : 1S	5R : 11S		
F ₂ Bond × Hawkeye	23	73	55	102	6.53
F ₂ Bond × S.D. 334	11	9	20	151	1.94

^a R = resistant; S = susceptible.

^b The χ^2 values at the 5 per cent and 1 per cent levels for 3 degrees of freedom are 7.82 and 11.34, respectively.

^c Totals for 10 lines, each of which segregated 9R: 7S in the F_2 seedling test.

^d χ^2 values for the ratio of 1 resistant: 1 segregating (3 resistant: 1 susceptible): 2 segregating (9 resistant: 7 susceptible): 12 susceptible.

of Bond × Hawkeye consisted of 10 plants from each of 22 F_2 lines which segregated in a ratio of 9 resistant to 7 susceptible in the F_2 seedling tests. For all crosses, with the exception of Bond × S.D. 334, the segregation was 1 resistant: 4 segregating (3 resistant: 1 susceptible): 4 segregating (9 resistant: 7 susceptible in the seedling stage and 5 resistant: 11 susceptible in the adult stage): 7 susceptible. The agreements between the observed and expected ratios were good except for S.D. 334 × Bond (population 2974) where too many F_2 lines were completely susceptible and too few were found in the segregating classes.

The segregation found can be explained by the hypothesis offered by

Hayes, Moore, and Stakman (1) or that by Torrie (7), although the genotype patterns would be quite different. Hayes *et al.* (1) explained the segregation of crosses involving Bond by 2 complementary factors carried by Bond which in the heterozygous condition resulted in somewhat lower resistance than when homozygous. Torrie (7), for the cross Iowa No. 444 \times Bond, suggested the presence of two factor pairs, S a factor for crown-rust resistance and I a factor which partly inhibits the expression of S and in which the masking effect of the inhibitor is greater in the adult plant stage in the field than in the seedling stage in the greenhouse. This would explain the difference in the double heterozygous plants which were classified as susceptible in the adult plant stage and resistant in the seedling stage. Since the double heterozygous plants compose one-quarter of the F_2 population the expected segregation in the F_2 and of the double heterozygous plants in the F_3 would be 9 resistant : 7 susceptible in the seedling stage and 5 resistant : 11 susceptible in the adult stage.

The Bond \times S.D. 334 progeny segregated somewhat differently than the other crosses. The F_2 segregated into the ratio of 15 susceptible : 1 resistant. The F_3 , which was grown in the field in both 1938 and 1939 and in the greenhouse during the winter of 1938-39, segregated approximately as follows: 1 resistant : 1 segregating (3 resistant : 1 susceptible) : 2 segregating (9 resistant : 7 susceptible) : 12 susceptible.

The F_3 reaction of Bond \times Hawkeye to races 3 and 6 of *Puccinia coronata avenae* was the same as the reaction to the composite inoculum.

Inheritance of Stem-Rust Reaction

The segregation for stem-rust reaction and tests of goodness of fit for the F_2 and F_3 data are given in tables 3 and 4, respectively. In the adult plant stage F_1 plants of all the crosses studied were resistant. This is in agree-

TABLE 3.—*Stem-rust reaction for the F_2 progenies of Bond (Avena byzantina) crossed with several varieties of A. sativa, and tests of goodness of fit to the 3 resistant : 1 susceptible ratio*

Cross and population	F_2 reaction		χ^2 values ^a
	Resistant	Susceptible	
	No. of plants	No. of plants	
Bond \times Hawkeye	184	69	0.68
Hawkeye \times Bond	98	30	0.17
Bond \times S.D. 334	133	58	2.91
S.D. 334 \times Bond (2974)	90	25	0.67
S.D. 334 \times Bond (2975)	88	21	1.88
S.D. 334 \times Bond (2976)	102	37	0.29
Hancock \times Bond (2987)	94	11	11.88
Hancock \times Bond (2988)	85	19	2.51
Hancock \times Bond (2989)	70	23	0.002
C.L. 3350 \times Bond (2983)	93	31	0.00
C.L. 3350 \times Bond (2984)	96	29	0.23
C.L. 3350 \times Bond (2985)	92	37	0.96

^a The χ^2 values at the 5 per cent and 1 per cent levels for 1 degree of freedom are .34 and 6.64, respectively.

TABLE 4.—*Stem-rust reaction for the F_2 progenies of Bond (*Avena byzantina*) crossed with two varieties of *A. sativa*, and tests of goodness of fit to the 1 resistant : 2 segregating : 1 susceptible ratio*

Cross	F_2 reaction			χ^2 values ^a
	Resistant	Segregating	Susceptible	
	Number	Number	Number	
Bond \times Hawkeye ..	59	127	67	0.51
Bond \times S.D. 334 ..	49	87	55	1.85

^a The χ^2 values at the 5 per cent and 1 per cent levels of significance for 2 degrees of freedom are 5.99 and 9.21.

ment with the results reported by Humphrey and Coffman (2) and by Torrie (7). The F_2 data, for the 6 crosses, gave good fits to a 3 resistant : 1 susceptible ratio with the exception of Hancock \times Bond (population 2987). The poor fit in the latter case was due to too many plants being classified as resistant. Since the other two populations of Hancock \times Bond showed good fits to the 3:1 ratio and since the stem-rust epidemic was light in certain portions of the field during 1939, the poor fit obtained for population 2987 is probably the result of certain susceptible plants escaping infection.

The F_2 segregation of the crosses Bond \times Hawkeye and Bond \times S.D. 334 grown in the field in 1939, gave good fits to the ratio of 1 resistant : 2 segregating (3 resistant : 1 susceptible) : 1 susceptible. These results substantiate the F_2 segregation.

Inheritance of Kernel Characters

The inheritance of several kernel characters, including number and length of basal hairs, type of basal articulation, rachilla attachment, lemma color, awnedness, and twisted black base of awn, were studied for the crosses Bond \times Hawkeye and Bond \times S.D. 334. Descriptions of the parental varieties, for the characters studied, are given in summary form in table 5.

The F_1 of the two crosses showed partial dominance of the grain characters of the *Avena sativa* parents. The data in table 6 give the modes of inheritance and tests of goodness of fit for the characters studied based on the segregation of the F_2 lines. In the cross Bond \times Hawkeye all segregations gave good fits to the 1 : 2 : 1 ratio including that for awn type. Since

TABLE 5.—*Kernel characters of Bond, Hawkeye, and S.D. 334*

Character	Bond C.I. 2733	Hawkeye C.I. 2464	S.D. 334 C.I. 2884
Basal hair length	Medium long	Short	Short
Number of basal hairs	Many	Few to absent	Few
Basal articulation	Sucker	Solid	Solid
Rachilla attachment	Secondary	Primary	Primary
Lemma color	Light reddish brown	Yellow	Yellowish white
Degree of awnedness	Weak awn present	Partly awned	Heavy
Twisted black base of awn	Absent	Absent	Absent

neither parent possessed awns with a twisted black base and since very few plants were found to be as weakly awned as *Hawkeye*, the exact parentage of the male parent of this cross may be questioned. With this exception, however, all segregations were as expected.

In the cross *Bond* × *S.D. 334* segregation of the F_2 lines for all characters, with the exception of basal articulation, occurred in the ratio of 1 byzantina complex : 8 segregating : 7 sativa complex. Due to various degrees of inter-

TABLE 6.— F_2 segregation for several kernel characters of *Bond* × *Hawkeye* and *Bond* × *S.D. 334*, and tests of goodness of fit to the 1:2:1 ratio for *Bond* × *Hawkeye* and to the 1:8:7 ratio for *Bond* × *S.D. 334*

Class and character	<i>Bond</i> × <i>Hawkeye</i>		<i>Bond</i> × <i>S.D. 334</i>	
	Number of lines	χ^2 values	Number of lines	χ^2 values
Basal hair length:				
Long	58		10	
Segregating	132	0.66	96	0.14
Short	63		79	
Basal hair number:				
Many	60		12	
Segregating	131	0.34	87	0.17
Few	62		80	
Type of basal articulation:				
Sucker	60		12	
Segregating	128	0.23	42	0.49 ^b
Solid	65		125	
Rachilla attachment:				
Secondary	63		13	
Segregating	124	0.18	93	0.79
Primary	66		73	
Lemma color:				
Reddish brown	62		12	
Segregating	125	0.17	91	0.15
Yellow to white	66		76	
Awning:				
Weak awned	63		14	
Segregating	122	0.55	87	0.78
Heavy awned	63		78	
Twisted black base of awn:				
Absent	65		13	
Segregating	126	0.07	90	0.37
Present	62		76	

^a The χ^2 value at the 5 per cent and 1 per cent levels for 2 degrees of freedom are respectively 5.99 and 9.21.

^b χ^2 value for the 1:4:11 ratio.

mediacy no attempt was made to differentiate between the segregating lines. The segregation suggests the presence of complementary factors, both present in a dominant homozygous condition in *S.D. 334* and carried in a recessive condition in *Bond*.

The segregation for basal articulation occurred in a ratio of approximately 1 byzantina : 4 segregating : 11 sativa. In the classification of this character both in the F_2 and F_3 the plants of intermediate type of basal articulation approached very closely the articulation type found in the sativa

parent. Since segregation for this character was not clear-cut, many plants which were intermediate were placed in the sativa complex. This would result in some of the segregating F_3 lines being classified as homozygous for the sativa complex. This is probably the reason for the different ratio found in the F_3 for this character as compared to that found for the other characters studied.

The data in table 7 show the linkage intensities between the kernel characters for the cross Bond \times Hawkeye, which were calculated from the F_2 and the F_3 data by the method of maximum likelihood described by Immer (3). Genes for all kernel characters studied were located on the same chromosome.

In the cross Bond \times S.D. 334, the inheritance of each kernel character was

TABLE 7.—Linkage intensities between genes for several kernel characters in the cross Bond \times Hawkeye

Character	Linkage intensities with standard error					
	Awne- ness	Twisted black base of awn	Basal articu- lation	Hair number	Rachilla attach- ment	Basal hair length
Twisted black base of awn	8.7 \pm 1.2					
Basal articulation	19.4 \pm 1.9	11.7 \pm 1.5				
Hair number	26.5 \pm 2.3	18.4 \pm 1.8	6.9 \pm 1.1			
Rachilla attachment	30.8 \pm 2.5	24.4 \pm 2.2	20.1 \pm 2.0	17.7 \pm 1.7		
Basal hair length	42.1 \pm 2.9	38.9 \pm 2.8	32.2 \pm 2.6	25.6 \pm 2.3	22.3 \pm 2.2	
Lemma color	58.7 \pm 3.4	50.0 \pm 3.0	47.8 \pm 3.1	46.4 \pm 3.0	44.0 \pm 3.0	26.8 \pm 2.3
	tbb	ba	hn	ra	hl	lc
	8.7	11.7	6.9	17.7	22.3	26.8

Chromosome map showing linear order of genes*

* a = awniness; tbb = twisted black base; ba = basal articulation; hn = hair number; ra = rachilla attachment; hl = hair length; lc = lemma color.

digenic. Chi-square tests of independence and association showed that the genes governing all the characters were closely linked. The P values in all cases were considerably less than 0.01.

Inheritance of Earliness of Heading

The distribution of the F_3 lines of the crosses Bond \times Hawkeye and Bond \times S.D. 334 and parental varieties for number of days from emergence to heading are given in figure 1. The distribution of the F_3 lines of Bond \times Hawkeye show that approximately one-third of the lines were earlier than Bond, and one-third as early as Bond. Seven of 253 lines studied were as late as Hawkeye, which is approximately one sixty-fourth of the entire population. The segregation found suggests that at least 3 factor pairs are involved and that Hawkeye, the late parent, possesses genes for earliness of heading.

The distribution of the F_3 lines of the cross Bond \times S.D. 334 show that approximately one-third were as late as the late parent, S.D. 334, and one-quarter as early as the early parent, Bond. The distribution suggests that at least 1 main factor pair and possibly 1 or more modifying factors are

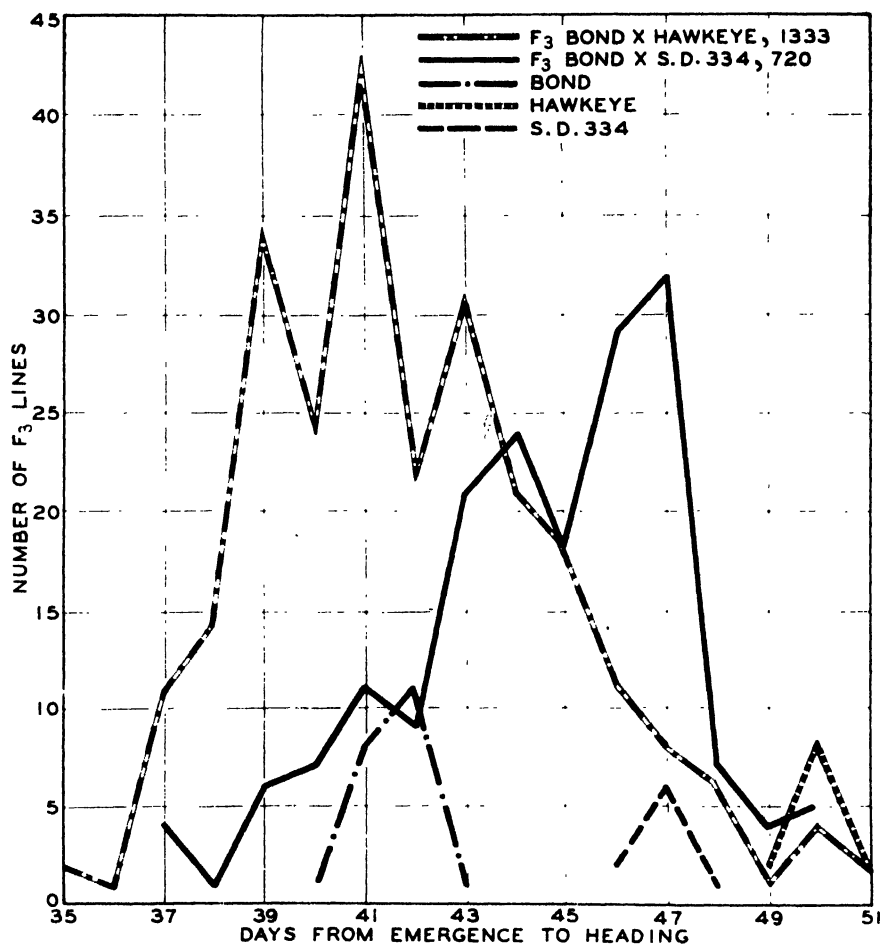


FIG. 1. The distribution of days from emergence to heading of F_3 lines for the crosses Bond \times Hawkeye, Bond \times S.D. 334 and parental varieties.

involved. A few lines earlier and later than either parent suggest the possibility of transgressive segregation.

SUMMARY

A study was made of the mode of inheritance of reaction to crown rust, *Puccinia coronata avenae*, and stem rust, *P. graminis avenae*, several kernel characters, and earliness between crosses involving several varieties of *Avena sativa* and Bond, *A. byzantina*.

The segregation found for crown-rust reaction in all crosses, save

Bond \times S.D. 334, can be explained by either the hypothesis of two complementary factors carried by Bond as suggested by Hayes *et al.* (1), or that of two factor pairs, S a factor for crown-rust resistance and I a factor which partly inhibits the expression of S as suggested by Torrie (7). Plants heterozygous for both factor pairs were more susceptible in the field than in the greenhouse.

A single factor pair with dominance of resistance governed stem-rust reaction.

In the cross Bond \times Hawkeye the characters of basal hair length and number, basal articulation, rachilla attachment, lemma color, awnedness and twisted black base of awn were monogenic and linked. In the cross Bond \times S.D. 334 the same kernel characters were digenic and linked.

A partial dominance of earliness of heading was found in the cross Bond \times Hawkeye, while in the cross Bond \times S.D. 334 lateness of heading was dominant over early heading.

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PHYTOPATHOLOGICAL NOTES

*Experiments with New Organic Fungicides for Control of Apple Scab and Brooks' Fruit Spot (Phoma pomii).*¹—Cooperative apple-scab-control experiments were conducted in Delaware on the Red Delicious and Rome varieties of apples and in New Jersey on the Rome variety during the 1944 growing season. Several of the newer organic fungicides were tested in dosage series. The test on the Rome trees in the Delaware experiment was begun after scab had become established in the planting.

In the Delaware experiments, U. S. R. No. 604 (2,3-dichloro-1,4-naphthoquinone) at 1 and 1½ pounds per 100 gallons and Puratized N5-X (phenyl mercuri triethanol ammonium lactate) at 1-1,000 (1-8,600 active ingredient) and at 1-2,000 (1-17,200 active ingredient) equalled liquid lime-sulphur at 1½ gallons per 100 gallons of water for scab control. Fermate (ferrie dimethyl dithiocarbamate) at 1½ and 2 pounds per 100 gallons, Fermate at ½ pound plus ½ the recommended quantity of Micronized Sulfur, No. 604 at ¾ pound or at ¾ pound plus ZnSO₄-lime ½-1, and Isothan Q-15 (lauryl isoquinolinum bromide) at 1-1,000 (1-5,000 active ingredient) equalled the standard wettable sulphurs in providing protection against scab. He 175 (disodium ethylene bisdithiocarbamate) as used (0.45 to 1.8 pounds per 100 gallons) either alone or in combination with zinc sulphate-lime failed to provide satisfactory control of apple scab.

The scab control in the New Jersey experiments was in the same order as those in Delaware, with the exception that No. 604 at the 1-pound dosage and Isothan Q-15 dropped to slightly below the efficiency of a wettable sulphur.

In an experiment at Cheswold, Delaware, designed to compare the eradicant value of Puratized N5-X with liquid lime-sulphur, Rome trees with scab on the foliage and fruit were either left unsprayed or were sprayed with lime-sulphur or Puratized N5-X. Puratized N5-X, at 1-1,000 and 1-2,000, had eradicative properties at least equal to those of lime-sulphur: only 9.5 and 10.5 per cent of fruit was infected after Puratized treatment, 17 per cent after lime-sulphur spray, and 97 per cent on trees without treatment.

In experiments in New Jersey during the last 3 years on the Stayman variety, Thiosan (tetramethyl thiuram disulphide) at ¾ pound in 100 gallons, Fermate at ¾ pound, and lead dimethyl dithiocarbamate at ¾ pound have given excellent control of Brooks' fruit spot (Table 1), when used at the 17-, 27-, and 37-day (after petal fall) applications. Trees sprayed with these materials, used with or without oil, have been remarkably free from spray injury. In 2 of the 3 years that Fermate has been used during the early cover spray period, lime in combination with this material has not produced any more russetting than has Fermate without lime. In 1943, however, the combination of lime and Fermate was more phytocidal, as judged by fruit russetting, than was Fermate alone. Where No. 604 was used with

¹ Journal Series paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Plant Pathology.

TABLE 1.—Control of Brooks' fruit spot on Stayman apple at Vincentown, New Jersey, in 1942 and 1943

Pounds of materials used in 100 gallons of water ^a	1942	1943	
	Fruit free from	Fruit free from	
	Fruit spot	Fruit spot	Russett
	Per cent	Per cent	Per cent
Lead dimethyldithiocarbamate, lime, 2-3	99.0
Tetramethyl thiuram disulphide, lime, 2-3	98.7
Fermate, lime, 2-3	98.7
do, 1½	98.5	97.6
do, ½	97.5	94.7
do, lime, ½-3	98.1	84.6
Bordeaux, ½-4	94.9	87.3	90.7
do, ½-4 + Coposil, ½	97.1	96.8	92.1
Lime, 3	75.0	61.6	97.4
Micronized Sulfur, lime, 4½-3	75.8	62.5	92.6
Check, not sprayed	12.9	5.6	94.6

^a All spray combinations carried 3 lb. of lead arsenate in the 17-day and 2 lb. in the 27- and 37-day applications. A summer oil emulsion was used at the rate of 0.75 per ce in the diluted spray at the 27- and 37-day applications.

oil, excessive injury occurred. Oil was used with Puratized N5-X, Isoth. a Q-15, and He 175 during 1945 without increasing the injury to apple foliage or fruits. The combination of Puratized N5-X with oil was very toxic, however, to poison ivy growing under the trees. In Delaware, No. 604 and oil produced excessive injury to Rome, whereas injury was slight with Puratized N5-X and oil.—ROBERT H. DAINES, Associate Plant Pathologist, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, New Jersey, and S. L. HOPPERSTEAD, Associate Research Professor in Plant Pathology, Delaware Agricultural Experiment Station, Newark, Delaware.

*Ring-Rot-Like Symptoms Produced by Soft-Rot Bacteria in Potato Tubers.*¹—In 1944, many potato fields in Minnesota were flooded by heavy rains at harvest time, resulting in various types of decay, both in the field and in storage. While the effect of water on tuber decay was being studied in the laboratory, symptoms similar to those of bacterial ring rot (*Corynebacterium sepedonicum*) were observed. (See figure 1, B.) Soft-rot bacteria (*Erwinia carotovora*) appeared to be the cause of the symptoms which had not been seen previously, or at least not recognized, in the field or on stored tubers from flooded fields.

Certified seed stock of the Cobbler variety, which had been stored for approximately seven months under optimum storage conditions, was used in the original experiment. The tubers were submerged in water held at the constant temperatures shown in figure 2, and were removed from the water and examined at the time intervals indicated in the same figure.

¹ Paper No. 2252, the Scientific Journal Series, Minnesota Agricultural Experiment Station.

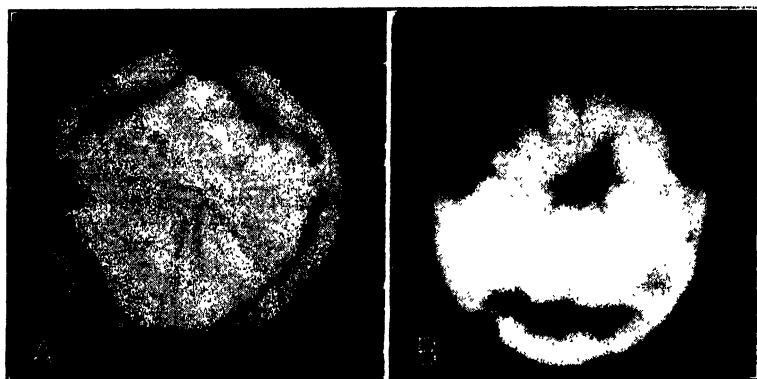


FIG. 1. A. Ring-rot-like symptoms produced by soft-rot bacteria in tuber of Cobbler; B. Bacterial-ring-rot symptoms produced by *Corynebacterium sepedonicum* in the same variety.

Figure 1, A, illustrates the symptoms, which resemble those of ring rot in that the tissue in the region of the vascular ring decays and this causes the cortex to separate easily from the medulla. In the earlier stages of breakdown the infection in the vascular area radiates away from the stem end and from the eyes of the tuber, indicating that infection occurs at these points. When a slight pressure is applied to a cut tuber, a white to cream-color ooze exudes from the infected portion of the vascular elements. This ooze, however, is lighter than that associated with ring rot and is creamy rather than cheesy in consistency. A water-soaked area extends on both sides of the vascular elements as seen in figure 1, A. The relation of temperature and length of time flooded to the occurrence of this type of vascular decay is indicated in figure 2.

The experiment was repeated, using again the Cobbler variety from the same source, certified seed stock of Bliss Triumph which had been stored for the same period of time, and immature Bliss Triumph tubers from Texas which were obtained on a local early spring produce market. The results with tubers of the Cobbler variety were the same as those reported in figure 2. The stored Triumph tubers responded similarly except that they

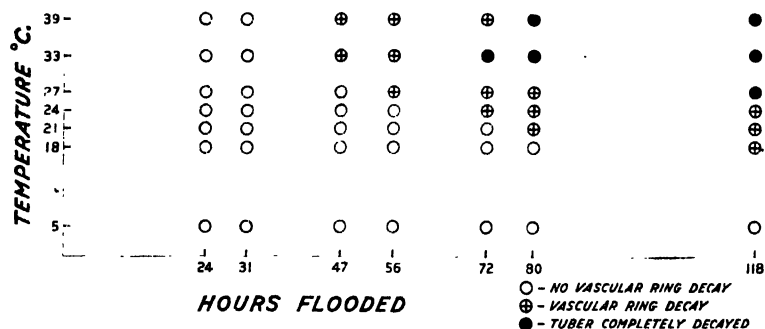


FIG. 2. Relation of temperature and length of time flooded to the occurrence of ring-rot-like symptoms in tubers of Cobbler.

appeared to be slightly more susceptible to infection than the Cobbler. The symptoms appeared in the new Triumph tubers 48 hours after being observed in the stored Triumph tubers.

All isolations from affected tubers have yielded Gram-negative bacteria of the *Erwinia* soft-rot group. Thus it appears that soft-rot bacteria, in potato tubers which are subjected to the conditions described, may produce symptoms resembling those produced by the bacterial ring-rot organism.—R. S. DAVIDSON, University Farm, St. Paul, Minnesota.

Sclerotium Rot of Potato Seed Pieces.—*Sclerotium rolfsii* Sacc. was found attacking potato seed pieces in a field near Hastings, Florida, in March, 1945. Cabbage had been grown in this field during the winter and after that crop was removed the field was planted to potatoes of the Sebago

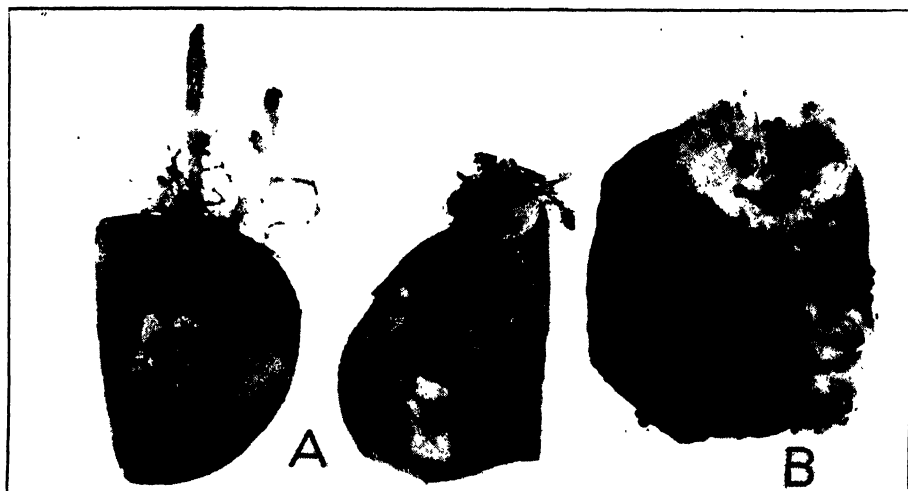


FIG. 1. Mycelium of *Sclerotium rolfsii* growing in seed pieces which had been naturally infected in the field (A), and typical mycelium and sclerotia of *S. rolfsii* on a naturally infected piece held in a moist chamber for 5 days (B).

variety on February 15. The soil was extremely dry when the potatoes were planted and it remained dry until the latter part of March as no rain fell to wet it and the field was not irrigated. The seed germinated poorly. Some pieces failed to produce sprouts or roots while others formed a few roots and clusters of sprouts similar to those seen on sprouted tubers in storage bins. The tips of most sprouts shriveled, died and turned brown in the hot, dry sand as soon as they emerged from the soil and were exposed to the sun. When seed pieces were examined, one month after planting, approximately 4 of each 10 were affected with a rot which originated on the cut sides of the pieces. A white mycelium resembling that produced by *S. rolfsii* was found in decayed holes in the pieces. Mycelium grew very rapidly on decaying pieces placed in a moist chamber (Fig. 1, A), and typical sclerotia of *S. rolfsii* formed on one piece kept in the chamber for 5 days (Fig. 1, B).

Bits of the mycelium of the organism were taken from the rotting pieces and placed in contact with healed and nonhealed cut surfaces of seed pieces which were then planted 2 inches deep in sand in 8 flower pots. Four pots were watered and four received no water. When examined 7 days later, small decayed areas were found in inoculated pieces in dry soil, but the pieces in wet soil had been almost completely destroyed by rot. Characteristic mycelium of *S. rolfsii* was present on pieces removed from wet soil and when one of these was held in a moist chamber for one week, the fungus produced abundant sclerotia. Mycelium and sclerotia also were found in the dirt and on inoculated pieces left in dry soil for 2 weeks. Healed seed pieces became infected as soon as the freshly cut pieces when the organism was placed in contact with the cut surfaces. The flesh of affected pieces was soft and white in decayed areas but no offensive odor was present.

A sclerotium rot of tubers caused by *S. rolfsii* occurs throughout the southern states^{1, 2, 3} but, so far as known, this is the first report of the natural occurrence of sclerotium rot of seed pieces in the field. Edson and Shapovalov⁴ recorded rotting of seed which had been inoculated with *Sclerotium rolfsii* prior to planting, but they did not report finding any seed pieces which had been naturally infected with the disease in the field.

The causal organism is being reported under the name of the vegetative stage because no basidial stage was found associated with the disease and no very clear-cut characters have been reported for separating *Corticium rolfsii* (Sacc.) Curzi and *C. centrifugum* (Lev.) Bres. in the vegetative stage.—A. H. EDDINS and EDDMAN WEST, Agricultural Experiment Station, University of Florida, Gainesville, Fla.

¹ Eddins, A. H. Diseases of plants in the United States. U. S. Dept. Agr., Plant Dis. Rptr. 25: 354. 1941. Suppl. 119: 241. 1939. Suppl. 128: 290. 1940.

² McClintock, J. A. A tuber rot of Irish potatoes. Tenn. Agr. Exp. Sta. Circ. 32. 1930.

³ Weber, G. F. Southern blight, *Corticium rolfsii*, on potato tubers. Phytopath. 33: 615-617. 1943.

⁴ Edson, H. A., and M. Shapovalov. Parasitism of *Sclerotium rolfsii* on Irish potatoes. Jour. Agr. Res. [U.S.] 23: 41-46. 1923.

LEON HATCHIG LEONIAN

1888-1945

C. R. ORTON

Dr. Leon Hatchig Leonian was born at Van, Armenia, February 27, 1888, and received his secondary education there. He emigrated to the United States at the age of 20, where he worked for a few years in New York and in Detroit.

He was graduated from the University of Kentucky with the B.S. degree in 1916 and from the University of Michigan with the degree of M.S. in 1917. Following a year as Assistant Research Horticulturist at Clemson College and a period as Assistant Professor of Botany and Plant Pathology in the New Mexico State College and Experiment Station, he returned to the University of Michigan. There he studied Mycology under Kaufmann and received the Ph.D. degree in 1922. That year he was appointed Assistant Plant Pathologist in the College of Agriculture and Experiment Station at West Virginia University. Here he became Professor of Mycology and Mycologist in the Experiment Station in 1936, the position he held to the time of his death, June 7, 1945.

Professor Leonian's early work was devoted to the study and control of plant diseases, particularly those caused by the downy mildews and by the *Fusaria*. His training and his experiences with the behavior of fungi led him into more detailed studies of the physiology of fungi, studies which became his major research program during his last fifteen years. His work on the influence of growth and sexuality factors in fungi and on growth factors for bacteria was productive of a long series of publications in ranking scientific journals.

Dr. Leonian was highly individualistic, always interested in living organisms, their habits of development and reproduction, their growth and metabolism. He was never interested in dead specimens. He abhorred the herbarium. To him taxonomy by the accustomed method of studying herbarium specimens was a great waste of time. His ultimate aim was to discover as many potentialities as possible of those living organisms in which he was particularly interested. His later association with Dr. V. G. Lilly enabled him to delve deeper into the field of mineral and vitamin metabolism of several groups of lower organisms—work which was productive of several new contributions to science.

As an avocation he became interested in the breeding of delphiniums, day lillies, and oriental poppies, achieving outstanding results from hybridization. Seeds from his "Lyondel Gardens" justly received international acclaim. With his ability as a speaker and as a flower breeder, he was much in demand as a speaker at garden club conventions in a several-state area. For many years he edited the *Delphinium Year Book* and in 1935 published "How to Grow Delphiniums" in book form.



LEON HATCHING LEONIAN
1888-1945

As a teacher his knowledge, his ready wit, and his sympathetic understanding of their problems made him a favorite with his students. In tribute to his interest in the welfare of students, his friends and associates have established a Student Loan Fund in his memory.

A large host of friends and associates will remember for their life-time his frank ways and genial smile.

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DIPLODIA ROT OF ONIONS

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(Accepted for publication October 10, 1945)

A dry or leathery rot of Texas-grown Crystal Wax onions caused by a species of *Diplodia* was first found on the Chicago market in June, 1938 (5), and on the New York, N. Y., market during the following season. The disease has been observed from time to time each year since then and was unusually prevalent on both markets during 1944. The decay has been found only on Texas-grown onions and only on the white varieties. Colored onions shipped from Texas during June do not show the disease. Although as high as 25 per cent of some lots of white onions have been affected, ordinarily the losses from *Diplodia* rot are not of great economic importance. However, the black discolorations produced are considered serious blemishes on white onions and the market value is reduced out of proportion to the actual decay present.

On market onions the characteristic symptom of this disease is silvery-gray to black discoloration of the outer dry scales about the upper half of the bulbs (Fig. 1, A, B, C). Occasionally the dry scales all over the onion are discolored (Fig. 1, F, G). In many onions dead and dying portions of the outer fleshy scales are also invaded and become black and leathery (Fig. 1, B, D) but the succulent living part of these scales is not invaded. In the more advanced stages black pycnidia singly or in groups of two or three are visible on dead scales (Fig. 1, C, F, G). Much of the mycelium that causes the dark discoloration is on the surface of the outer dry scale and between this scale and the first fleshy scale (Fig. 2, A, B). No decay of the internal fleshy scales has ever been observed.

Isolations from the discolored dry outer scales and from the dying tips of the first fleshy scales at the neck have consistently yielded a species of *Diplodia*. The spores produced are for the most part one-celled and hyaline (Fig. 2, C), but with age a high proportion of them become brown, striated, and 2-celled (Fig. 2, D). Spores obtained from pycnidia on onions and those produced in pure cultures usually range from 10.2 to 17.7 \times 20.0 to 28.4 μ , averaging 12.8 \times 23.6 μ . Neither in pure culture nor in diseased onion tissue has a perfect stage been observed.

On potato-dextrose-agar plates the minimum temperature for growth of the fungus was 50° F.; the optimum 85°; and the maximum 104°.

In plate cultures the onion *Diplodia* was compared with 12 other isolates of *Diplodia* from six different hosts, namely, avocado, cocoanut, orange, peanut, sweet potato, and watermelon. Although variations in character of growth and in spore measurements were observed, in no instance did they

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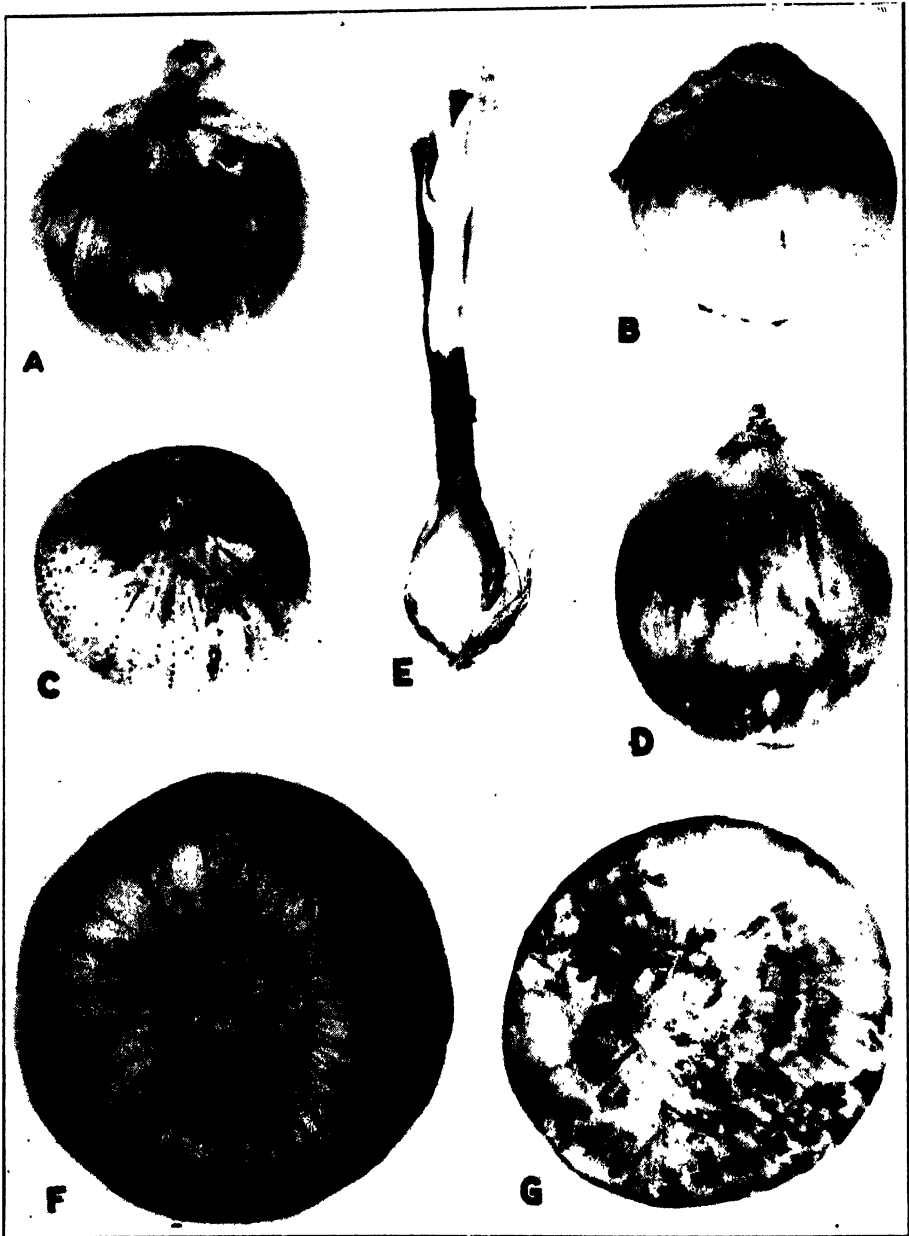


FIG. 1. *Diplodia* rot of onion. A, B, C. Naturally infected white onions with typical discoloration and decay as noted on the market. D. Leathery type of decay on dying outer fleshy scales of naturally infected white onion. E. Discoloration of dry scales at neck of onion seedling resulting from artificial inoculation. F, G. Advanced stages of discoloration of outer scales of naturally infected white onion. Pycnidia are abundant in C, F, and G.

appear to be of sufficient importance to indicate that distinct species were involved.

The onion *Diplodia* has been proven pathogenic to sweet potato, apple, and orange by means of inoculation experiments. Apples inoculated through a side wound developed an extensive brownish-black lesion within a week and ultimately the whole fruit was converted into a black, moderately firm mummy. Pyrenidia were formed in the peel and the internal tissues were full of large hyphae typical for *Diplodia*. Stem-end inoculations of oranges with the onion *Diplodia* produced typical stem-end-rot symptoms identical with those produced by *D. natalensis* from oranges. In other cross-inoculation studies isolates of *Diplodia* from onion, watermelon, sweet potatoes, and

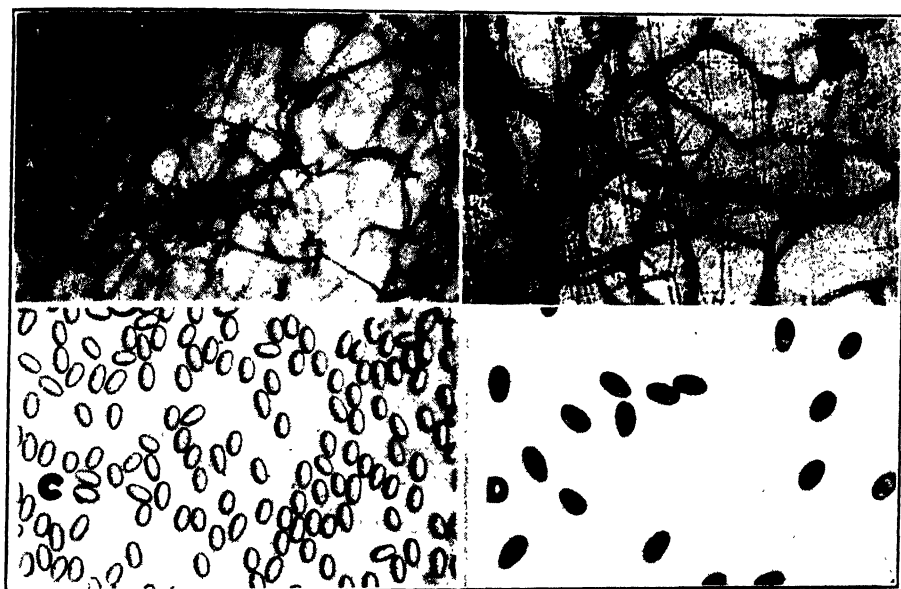


FIG. 2. *Diplodia natalensis* from onion. A and B. Hyphae on and between outer scales of white onion bulbs. 100 \times and 215 \times , respectively. C. Young 1-cell hyaline spores. 215 \times . D. Brown, striated 2 cell spores. 215 \times .

oranges proved pathogenic when inoculated in sweet potatoes. A dark brown, fairly firm, moist decay with a sharp line of demarcation between the diseased and healthy tissue, characteristic of Java Black Rot in sweet potatoes, was produced in each instance.

These results are in agreement with those of others (1, 2, 6, 7) who have shown that strains of *Diplodia* from a wide variety of crop plants are similar morphologically and produce similar symptoms when cross-inoculated on a number of different host plants.

On the basis of the present studies the writers consider the onion *Diplodia* to be a strain of *Diplodia natalensis* Pole-Evans.

The invasion of onion by *Diplodia* always appears to be limited to the dead outer scales and the dying fleshy scales at the neck and down the side

of the onion, thus indicating that the fungus is saprophytic or only weakly parasitic to onions. Some growth of the fungus was obtained by inoculating the dry scales of a young onion plant just above the bulb and holding it in a moist chamber at 75° F. for ten days (Fig. 1, E). The only growth ever obtained in the fleshy scales was in white peeled onions that had been dipped in boiling water long enough to kill the tissues of the outer scales. All attempts to produce decay by inoculating fleshy succulent scales of white, yellow, and red onions have failed. This is in line with observations of this disease on market onions.

In view of the fact that growth of this *Diplodia* seems limited to white

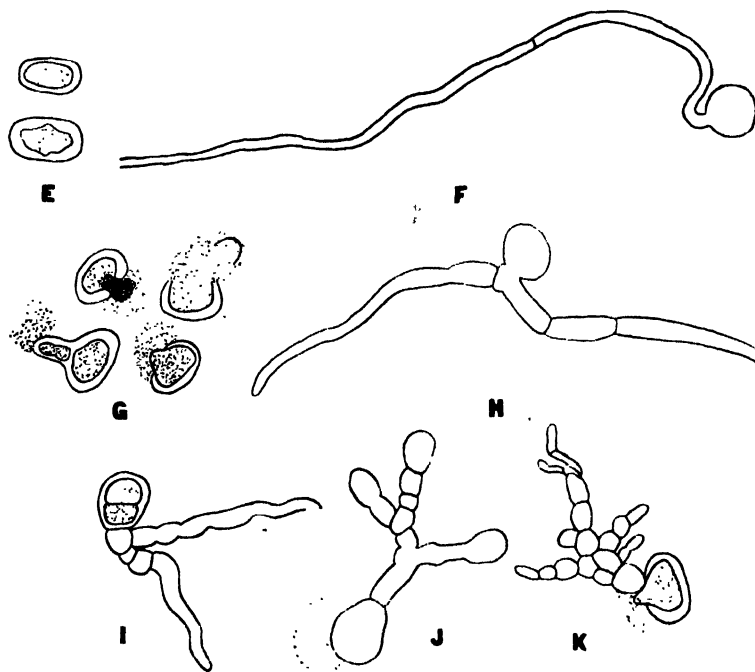


FIG. 3. *Diplodia natalensis* from onion. Camera lucida drawings (375 \times). E. Spores plasmolyzed by juice of fleshy scales. F. Normal germination of spore in water. G. Abnormal germination in a water extract of dry colored onion scales, with bursting of spores and extrusion of contents. H. Spore germinated in presence of dry white onion scales. I, J, and K. Abnormal germination in water extracts of yellow, brown, and red scales, respectively.

onions it was decided to check the reaction of this fungus to the color pigments in yellow, brown, and red onions to determine whether they were responsible for the apparent immunity of colored onions. To do this a suspension of *Diplodia* spores in sterile water was made and drops of the suspension were placed on sterile cover slips. Approximately 4 sq.mm. of colored or white onion scale was placed in these drops and the slips were then inverted over Van Tieghem cells. Drops of the spore suspension placed over Van Tieghem cells without the addition of onion scale were used as controls. All cells were allowed to stand from 18 to 24 hours at room temperature and

then counts of germinated spores were made. From counts of over 1,000 spores for each type of scale used, it was found that 92 per cent of the spores germinated in the control cells, 94 per cent in the presence of the white scale material (Crystal Wax), 66 per cent with the yellow scale (Yellow Bermuda), and 60 per cent with the red scale (California Red). The germ tubes produced by spores in the presence of the white scale tissue were normal (Fig. 3, H), whereas those produced in the presence of yellow and red scale tissue were abnormal. There were no normal germ tubes in the presence of any colored scale material and about half of the ungerminated spores burst and extruded their cytoplasm (Fig. 3, G). The germ tubes that were developed were shorter, thicker, more branched, and more septate than normal (Fig. 3, I, J, K). The germ tubes that developed in the controls and in the presence of white scale tissue were long and slender with few cross walls and little branching (Fig. 3, F, H). The reaction of this fungus to the pigmented onion scales is very similar to that of *Colletotrichum circinans* (Berk.) Voglino as reported by Walker and others (8, 9, 10, 11).

The effect of water extracts of pigmented onion scales on spore germination of *Diplodia* was tested in many experiments. Except for slight variations, the results obtained were usually similar to those just reported. However, it was noted that the degree of pigmentation (yellow, brown, or red) influenced the character and amount of germination. The more concentrated the pigment, the less the germination and the more abnormal the germ tubes produced. The water extract of dark scales of Australian Brown onions inhibited spore germination to a greater degree than the colored scales of any variety tested. It was also noted that the spores farthest from the scale particle in the hanging drop had more nearly normal germination and growth than those nearest it. The indications are, therefore, that some chemical substance associated with the pigment in the dry outer scales is the principal inhibiting agent for *Diplodia*. In view of the work of Link *et al.* (3, 4), it is assumed that this chemical is probably protocatechuic acid.

Since the internal fleshy bulb scales of neither the white nor colored varieties of onion are invaded by *Diplodia* it seemed desirable to test the effects of the presence of bits of fleshy scale of white and colored onions on spore germination. This was done by placing a 4-sq. mm. piece of fleshy scale in a hanging-drop water suspension of spores in Van Tieghem cells, and also by expressing the juice, diluting it in a spore suspension in sterile water, and observing in hanging drops. Although the spores germinated in the dilute juice exuding from bits of fleshy scales and in dilute juice extract of all varieties tested in the hanging drops in Van Tieghem cells, at higher concentrations of juice, germination was abnormal or totally inhibited and many of the spores were plasmolized (Fig. 3, E). In an experiment in which the freshly extracted juice from fleshy scales was used in a one to one dilution with a water suspension of spores 2 per cent germinated, whereas at a 1 to 5 dilution 7 per cent germinated; at 1 to 10, 10 per cent, and at 1 to 20, 62 per cent. In the controls 61 per cent germinated. The inability of

Diplodia spores to germinate normally in onion juice probably accounts for the fact that no invasion of fleshy wounds has been found in onions on the market.

In an attempt to determine whether or not the vapors from fleshy scales would inhibit growth of *Diplodia*, Petri-dish cultures seeded with spores were turned upside down and into the lid was introduced the freshly expressed concentrated juice of a red onion. Practically all of the spores germinated and a normal growth of *Diplodia* was produced within 24 hours, thus indicating that the growth of *Diplodia* is not inhibited by volatile substances given off by the juice of fleshy scales.

The hydrogen-ion concentration of water extracts from white, yellow, and red dry scales and of the fleshy inner scales of three varieties of onion (Crystal Wax, Yellow Bermuda, and California Red) was determined in order to ascertain whether acidity might be a factor in limiting the invasion of onion tissue by *Diplodia*. The dry outer scales of each variety listed had a pH value of 4.4 to 4.5. The juice of the fleshy scales was practically identical for all three varieties, ranging from pH 5.7 to 5.9. Cultures of the onion *Diplodia* grew well on potato-dextrose agar adjusted to pH 4.4 and 5.5 but developed slightly more slowly than on the same medium adjusted to pH 6.8. Growth on the medium at pH 4.4 was retarded only slightly more than at pH 5.5. Since there was little variation in the acidity of the dry scales or of the fleshy scales in the white susceptible variety in comparison with colored immune varieties of onion, and since the fungus grew well within the acid range found in these scales, these data indicate that the acidity of onion juice is not the primary factor in preventing *Diplodia* from becoming an active decay-producing organism in onions.

SUMMARY

A moderately serious market disease of Texas-grown white-skin Crystal Wax onions caused by *Diplodia natalensis* Pole-Evans is described for the first time. Colored-skin varieties of onions from Texas were not affected.

Only the dead outer scales and the dying parts of the outer fleshy scales of white onions are invaded. No decay of living fleshy scales of bulbs has been observed and all attempts at inoculation of such tissues have failed.

A chemical (probably protocatechuic acid) associated with the pigments in water extracts of the dry outer scales of colored varieties of onions proved toxic to the onion-*Diplodia* spores. Dry scales of white onions do not carry this toxic agent in sufficient quantity to interfere with the germination of the spores.

Determination of the pH of both the dry outer scales and the fleshy scales of white and of colored varieties of onion shows that acidity of these tissues is not the primary factor in limiting the pathogenicity of the onion *Diplodia* to white varieties.

The studies here reported and the market observations made during the past seven years indicate that although *Diplodia natalensis* is not strongly

pathogenic to onions, it may cause a great reduction in market value by producing slight decay and by blemishing the southern white onion crop.

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INFLUENCE OF TIME, RATE, AND DEPTH OF SEEDING ON THE INCIDENCE OF ROOT ROT IN WHEAT¹

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INTRODUCTION

Common root rot is by far the most widespread and destructive root disease of wheat in Manitoba. It attacks both seedlings and mature plants. Although *Helminthosporium sativum* P. K. and B. and certain species of *Fusarium* are considered the primary cause of common root rot, other soil-borne fungi are constantly associated with the disease in this Province. Furthermore, drought, poor nutrition, and other environmental factors are almost invariably associated with the disease in the field.

The loss to the wheat crop of Manitoba from common root rot is substantial. For the 10-year period 1928-1937, the average annual loss to this Province was estimated to be not less than 1,909,000 bushels, or an annual cash loss of \$1,336,000.00 for that period (2). This estimate, however, was based on the results obtained in experimental plots at Winnipeg and, when made, was recognized to be extremely conservative, as is now evident. On the basis of intensive systematic field surveys, Machacek (14) estimated that, for the 3 years, 1939 to 1942, the annual reduction in yield of wheat in Manitoba from common root rot was 12.1 per cent. This represented an average yearly monetary loss of \$3,827,000.00.

The simplest and most effective method of reducing the losses caused by common root rot is by the use of varieties in which resistance to the disease has been combined with other desirable qualities by the process of hybridization and selection. Although certain varieties of wheat are known to be much more resistant than others, the problem of breeding varieties possessing high resistance to root rot is a difficult one, and there is the possibility that varieties wholly resistant under all environmental conditions may not be obtained for a considerable time. In the meantime, the control of the disease must depend upon other preventive measures such as crop rotation, manurial treatments, and cultural practices.

The present paper is concerned with the influence of certain cultural practices on the control of root rot of wheat, and reports the results of field experiments, carried out from 1936 to 1944, to determine the relation between the date, rate, and depth of seeding of spring wheat and the incidence of root rot caused by *Helminthosporium sativum* and *Fusarium spp.* in seedling and adult plants. A preliminary report on these experiments has already appeared (10).

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EXPERIMENTAL METHODS

The field experiments were, for the most part, at Winnipeg, Manitoba. The soil at this station is classified as a heavy-textured black earth (4). It is a heavy clay, and although the surface soil is fairly uniform in texture the subsoil is quite variable. The studies on date and rate of seeding were made on summer-fallowed land, and those on depth of seeding were made on land that had been cropped continuously to wheat for a number of years.

The system of replication used in each experiment consisted of 4 or 6 randomized blocks, each date, rate, or depth of seeding occurring once in each block. Each plot consisted of 3 or more rows. The rows were spaced 1 foot apart. They were 18 feet long in the date and rate of seeding experiments, and 12 feet long in the depth of seeding experiments. Sufficient buffer rows were used in each plot to eliminate border effect. The seed was sown with a V-belt hand drill.

Data on plant emergence and seedling root rot were recorded about one month after the seed was sown. The methods, infection classes, and disease-rating formula used in obtaining these data were those employed by Greaney (8). Notes on the incidence of adult plant root rot were taken about 10 days before the plants ripened. These data were obtained as follows. The plants in one row of each plot were lifted from the soil and examined individually to determine the intensity of disease on each plant. In rating the plants for disease, 6 infection classes (0 to 5), indicative of the amount of basal lesioning and ranging from no lesioning to plants dead due to root rot infection, were used. The amount of disease in each plot was recorded as a root-rot rating.

This was calculated from the formula $R = \frac{S}{N \cdot 5} \cdot 100$, where R = root-rot ratings, S = sum of individual plant ratings, N = number of plants examined, and 5 = highest individual plant rating. Dividing S by N \cdot 5 and multiplying by 100 gives a rating which represents the amount of disease in terms of the maximum possible for the plot. In other words, a root-rot rating of 0 indicates no disease and 100 a maximum amount of disease.

Yield data were obtained by harvesting and threshing separately one row of each plot. The plant emergence, disease, and yield data of each experiment were treated statistically by the analysis of variance method, and the significance of the observed differences between dates, rates, or depths of seeding was determined.

EXPERIMENTAL RESULTS

Date of Seeding

Broadfoot (1), in a study of the effect of date of seeding on the incidence of foot rot of spring wheat caused by *Helminthosporium sativum* and *Fusarium spp.*, found that early-sown crops were more heavily infected with this disease than were late-sown crops. Robertson *et al.* (18) made a study of the relation of the date of seeding of winter wheat to dry-land foot rot and found that infection and severity of foot rot were greatest in the early

plantings. According to Russell (19) spring wheat, sown early, is more susceptible to the take-all (*Ophiobolus graminis* Sacc.) disease than if it is sown late. Kirby (13) states that late planting of winter wheat reduces the incidence of take-all.

An experiment designed to examine the influence of date of seeding on the development of *Helminthosporium-Fusarium* root rot was carried out at Winnipeg in 1936. Plots of Pentad wheat were sown at 4-day intervals from May 4 to June 12 (10 plantings). The experiment was repeated in 1937, 1938, and 1939. In these years the earliest planting was made on or near May 4. Thereafter, plots were sown at 4-day intervals until 10 plantings had been completed. The wheat variety Pentad was used in 1937 and 1938, and the variety Mindum in 1939. These varieties are susceptible to root rot. The seed used in each of the 4 experiments was heavily infected with *Helminthosporium sativum*. In 1936 and 1937 inoculum of *H. sativum*, prepared according to the methods described by Machacek and Greaney (15), was added to the rows at seed level as the seed was sown. No inoculum was added to the soil in 1938 and 1939.

Date of Seeding in Relation to Root-Rot Severity and Yield

The analyses of variance of the plant emergence, disease, and yield data of the date of seeding experiments are presented in table 1. A high degree of significance can be attached to the effects of date of seeding on plant emergence, disease development, and yield. For the disease data, the mean squares due to the interaction, dates \times years, were much greater than the error mean squares, and, for these data, the mean squares for dates significantly exceeded the mean squares due to the interaction. In other words, the relative effect of date of seeding on the development of root rot was the same in the 4 years of the experiment. Table 1 shows that the effect of years on all properties studied was very great, as would be expected. To economize space, the analyses of variance data for individual years are not given. For each year, however, the results of the analyses showed highly significant differences between seeding dates for all the properties measured.

TABLE 1.—*Analyses of variance of plant emergence, root rot, and yield data. (Date of seeding experiments, 1936 to 1939)*

Source of variance	Degrees of freedom	Mean squares			
		Percentage plant emergence	Seedling root-rot rating	Adult plant root rot rating	Yield
Replicates	5	200.46	50.76	75.02	91.76
Years	3	8,815.37*	61,047.50*	7,534.06*	2,530.22*
Error (a)	15	39.78	42.97	37.75	51.63
Dates of seeding	9	3,466.05*	670.09*	2,188.07*	870.23*
Dates \times years	27	201.98	145.66*	172.07*	81.25
Error (b)	180	168.82	34.36	41.74	75.12

Exceeds mean square error, 1 per cent level.

In table 2 are given the average field-plot data for the different dates of seeding. It is apparent that the date at which the seed was sown in 1936, 1937, 1938, and 1939 exercised a marked influence on the development of root rot. Delay in seeding increased the incidence of the disease in seedling and adult wheat plants. The results obtained in each of the 4 years indicated that late stands of spring wheat were more severely affected by root rot than were early stands. In 1939, for instance, the average seedling root-rot rating for Mindum wheat sown on May 11, May 19, May 27, June 4, and June 12 was 14, 17, 23, 29, and 31, respectively, the corresponding figures for the adult plant root-rot rating being 16, 27, 29, 35, and 42, respectively.

TABLE 2.—*Influence of date of seeding on plant emergence, yield, and on the incidence of seedling and adult plant root rot in wheat. Mean results of experiments at Winnipeg in 1936, 1937, 1938, and 1939*

Date of seeding	Average mean daily soil temperature ($^{\circ}$ F.) ^a	Percentage seedling emergence	Seedling root-rot rating	Adult plant root-rot rating	Yield (bu. per acre)
May 7	43.5	67.8	45.5	30.8	35.7
May 11	45.0	64.8	46.8	30.8	33.2
May 15	49.2	65.8	49.0	41.0	27.4
May 19	47.8	69.8	48.5	40.0	25.6
May 23	50.0	69.2	52.5	48.2	26.2
May 27	50.0	67.2	54.2	46.0	25.3
May 31	50.3	66.2	56.8	51.5	25.1
June 4	50.1	61.5	58.5	54.5	22.3
June 8	50.1	60.5	57.2	52.2	19.9
June 12	50.7	58.8	60.8	56.0	16.8
Necessary difference, 5 per cent level		7.9	3.6	4.0	5.3

^a Soil temperature at depth of 4 inches.

It is clear from table 2 that time of planting had a very marked and significant effect on yield. There was a progressive decrease in yield from the earliest to the latest date of seeding. The fact that yield was greatest in the early seedings, and that it is inversely related to disease severity, indicates that root rot was a factor of considerable importance in reducing yields in the late-sown plots.

The data presented in table 2 indicate clearly that time of seeding did not influence appreciably the number of plants that emerged from the soil, except in the case of plantings made after May 31. Very late planting (June 12) significantly reduced percentage plant emergence.

Soil Temperature in Relation to Root-Rot Severity and Yield

The extensive investigations of Dickson (3), McKinney (16), and Johnston and Greaney (12) have indicated that wheat plants are blighted most severely by root-rotting fungi at relatively high temperatures. This finding is in agreement with that of several other investigators. Garrett (6), in a review of the extensive studies on the relation of soil conditions to root-

infecting fungi, points out that soil temperature affects a soil-borne disease such as common root rot of wheat not only directly through its effect upon host resistance, and upon the causal fungi, but also indirectly by influencing the factor of microbiological antagonism to the parasites. From the foregoing statements it is evident that soil temperature has a profound influence on root-rot development. As root rot was found in the present experiments to be much more severe in late sown crops than in crops from early seedings, soil temperature data were studied to ascertain to what extent this variability in root-rot infection may be related to soil temperature conditions at different seeding dates.

The average soil temperature data for the experiments of 1936, 1937, 1938, and 1939 are presented in table 2. A comparison of the mean daily temperatures for the different dates of seeding shows that the lowest soil temperature occurred at the earliest seeding date (May 7), and that there was a gradual rise in temperature as the season progressed. There was, however, a greater rise in soil temperature at the second and third seeding dates (May 11 and May 15) than at later dates. The differences in mean daily soil temperature between individual dates of seeding after May 15 are so small that they probably have no significance.

The evidence presented earlier showed that date of seeding had a very marked and significant influence on the development of root rot, and on yield, and, from what has just been said, it is evident that a close relationship exists between soil temperature and date of seeding. It would, therefore, be expected that a close association would exist between soil temperature and the incidence of root rot, and between temperature and yield. That this is so is clearly brought out by a comparison of the data in table 2.

With a view of determining the relation of soil temperature to root-rot development and plant growth, a statistical study was made of the temperature, disease, and yield data of the experiments. In this study the original field data of the 4 experiments were used. When mean daily soil temperatures and seedling root-rot ratings for each planting date were correlated, a significant positive correlation coefficient of $+0.401$ was obtained, indicating an increase in seedling blight with an increase in soil temperature. A significant positive coefficient of $+0.692$ was also found between the degree of adult plant root-rot infection and temperature. These results indicate clearly that soil temperature was closely associated with the development of root rot. The higher the temperature at time of seeding the higher was the degree of infection.

A highly significant inverse relation ($r = -0.622$) was found between soil temperature and yield. In other words, low temperature (early seeding) was associated with high yield. When the yield and the disease infection data were correlated, definitely significant negative coefficients were obtained, indicating that high yield was associated with low disease incidence. The foregoing statistical results, together with the average data in table 2, indicate clearly that soil temperature affects yield, and that part of the reduction

in yield in the late plantings was due to the influence soil temperature had on the development of root rot.

In the final analysis of the field data the seedling and adult plant root-rot ratings were correlated. A significant positive correlation coefficient of +0.562 was obtained. This result indicates that the susceptibility of the wheat varieties Pentad and Mindum to *Helminthosporium-Fusarium* root rot does not depend on their stage of development. They were equally susceptible in the seedling stage and at the stage of growth just prior to ripening (Table 2). It can be concluded, therefore, that the seedling reaction is a reliable index of the reaction of adult wheat plants to root rot caused by *Helminthosporium sativum* and *Fusarium* spp.

Rate of Seeding

Guerrapain and Demolin (11), Föex (5), Peyronel (17) and others in Europe, and Kirby (13) in America, report that the development of the

TABLE 3.—*Analysis of variance of root-rot and yield data. (Rate of seeding experiments, 1938, 1939, and 1940)*

Source of variance	Degrees of freedom	Percentage plants diseased	Mean squares	
			Root rot rating	Yield
Replicates	5	150.41	58.16	153.18
Years	2	415.18*	387.60*	547.26*
Rates of seeding	5	1,292.27*	593.02*	4,053.00*
Rates \times years	10	64.64	27.54	20.33
Error	85	92.46	30.43	34.58

* Exceeds mean square error, 1 per cent level.

take-all disease of wheat is favored by dense sowing. Broadfoot (1), in studies on the effect of crop rotation and cultural practice on the development of foot rot of wheat, was unable to find any close relation between rate of seeding and the incidence of the disease. However, the belief is commonly held in Manitoba that there is a close association between rate of seeding and the incidence of common root rot in crops of spring wheat. In order to obtain definite information on this point field experiments were carried out in 1938, 1939, and 1940.

In each of these years seed of healthy Regent wheat was sown at 6 different rates, namely, 50, 100, 200, 300, 400, and 500 seeds to an 18-foot row. Three rows of each rate of seeding constituted a plot. Just before harvest, adult plant disease data were obtained in the usual way from the lateral rows of each plot. The center row of each plot was harvested for yield data.

The analyses of variance of the disease and yield data are given in table 3. For disease and yield the mean squares due to rates of seeding were significant. This means that density of sowing definitely affected the incidence of root rot and yield. The insignificant interactions for disease and yield indi-

cate that the effect of seeding rate on disease severity and on yield was the same in the 3 years of the experiment. The mean squares due to differences between years were all highly significant.

The variations in percentage of plants diseased, intensity of root-rot infection, and yield, due to rate of seeding, are shown in table 4. It is evident from this table that the incidence of root rot increased with an increase in the seeding rate. This is in accordance with general field observations in Manitoba and indicates that thinly-spaced wheat plants are less likely to be severely attacked by root rot than are thickly-spaced plants. Thus, the results of the experiments support the view that any cultural practice which tends to increase the vigor of individual wheat plants can be counted upon to reduce the amount of root-rot infection.

Table 4 shows that, in spite of a consistent increase in disease incidence, yield increased as the rate of seeding was increased from 50 to 400 seeds per

TABLE 4.—*Influence of rate of seeding on the incidence of root rot and yield in wheat in 1938, 1939, and 1940*

Rate of seeding per 18-foot row	Percentage of plants diseased				Adult plant root-rot rating				Yield (bu. per acre)			
	1938	1939	1940	Mean (1938– 40)	1938	1939	1940	Mean (1938– 40)	1938	1939	1940	Mean (1938– 40)
50	59	69	68	65.3	12	8	15	11.7	8.1	11.4	11.6	18.0
100	68	70	72	70.0	16	11	18	16.0	11.5	39.4	20.3	23.7
200	72	78	96	82.0	17	21	26	21.3	16.1	42.1	27.9	28.8
300	88	90	99	92.3	23	23	35	27.0	17.5	41.1	26.7	28.4
400	96	90	99	95.0	27	25	33	28.3	17.5	48.3	34.2	33.3
500	96	97	100	97.7	29	23	37	29.7	18.0	45.8	34.3	32.7
N.D. ^a	11.2	13.1	9.6	6.8	4.8	5.4	6.1	3.9	1.6	6.9	4.6	4.2

^a Necessary difference, 5 per cent level.

row. Above the 400-seed rate, however, there was no further increase in yield, a fact that is in agreement with the results of Machacek and Greaney (15) who found that individual plants in dense stands of wheat were less vigorous and tillered less than those in thin stands. This difference in vigor and tillering of plants accounts for the difference in their yielding ability, and also for the fact that very dense stands of wheat in the Winnipeg plots did not yield more than less dense stands. It is generally recognized, however, that, in Manitoba, the effects of rate of seeding on the development of root rot and on yield in wheat are likely to vary directly with the available soil moisture at seeding time, and also with the amount of rainfall from seeding time to harvest.

Depth of Seeding

Russell (19), in field experiments with the take-all disease of wheat, found that infection was increased by deep seeding. According to Broadfoot (1) depth of seeding had no significant effect on the incidence of *Helminthosporium-Fusarium* foot rot. To gain information as to the part

that depth of seeding plays in the development of common root rot, field studies were undertaken in 1943 and 1944.

In 1943, healthy seed of Thatcher and Red Bobs wheat was sown at 4 different depths in plots of infested soil at Winnipeg. The seeding depths used were 1 in., 2 in., 3 in., and 4 in. Each plot consisted of 3 rows, 1 foot apart, and 12 feet long. Two hundred seeds were planted in each row. Notes on the incidence of disease were taken on the center row of each plot in the usual way about 10 days before the plants ripened.

Evidence was obtained in 1943 which indicated that there was a positive relation between depth of seeding and the severity of root rot. Deep planting definitely increased the amount of root rot, and the increase was progressive. Taking 100 as the figure representing very severe root-rot infection, the mean values for plots of Thatcher and Red Bobs wheat sown at depths of 1 in., 2 in., 3 in., and 4 in. in 1943 were, respectively, 15.3, 21.6, 23.1, and 24.0.

TABLE 5.—*Analysis of variance of root-rot data. (Depth of seeding experiment, 1944)*

Source of variance	Degrees of freedom	Mean square	F value	5 per cent point	1 per cent point
Replicates	3	196.67	10.49	3.07	4.87
Varieties	3	219.58	11.71	3.07	4.87
Stations	1	2,320.67	123.77	4.32	8.02
Varieties \times stations	3	97.33	5.19	3.07	4.87
Error (a)	21	18.75			
Depths of seeding	2	410.60	51.84	3.19	5.08
Depths \times varieties	6	21.89	2.76	2.30	3.20
Depths \times stations	2	5.48			
Depths \times varieties \times stations	6	11.67	1.47	2.30	3.20
Error (b)	48	7.92			

In view of these results it was decided to repeat the experiment in 1944 using 4 varieties (Thatcher, Regent, Renown, and Red Bobs), and 3 seeding depths (1 in., 2 in., and 3 in.). This enlarged field experiment was conducted at Winnipeg and Morden, Manitoba.

The analysis of variance of the root-rot data of the 1944 experiment is given in table 5. From this it is evident that the mean square for depths of seeding is highly significant. On the other hand, the mean squares due to the interactions, depths \times stations and depths \times varieties, failed to reach significance at the 1 per cent point. In other words, the effect of the depths of seeding was the same at both stations, and for the 4 varieties. The mean squares due to varieties and to stations were significant. This means that a high degree of significance can be attached to the differences observed between varieties and stations.

The mean root-rot ratings for each variety and station, together with the mean values over all stations and varieties, are given in table 6. It is clear that depth of seeding had a very marked and significant effect on the devel-

opment of root rot. At Winnipeg and Morden the incidence of the disease increased with depth of seeding. Comparisons between individual depths of seeding for all varieties and stations show that the differences observed in disease incidence between the different seeding depths were statistically significant.

It is evident from table 6 that, in so far as the development of root rot was concerned, depth of seeding was more important in some varieties than in others. That is, the varieties did not react to depth of seeding to the same degree. For instance, deep seeding definitely increased the amount of root

TABLE 6.—*Influence of depth of seeding on the incidence of root rot in certain varieties of wheat in 1944*

Variety, and depth of seeding in inches	Root-rot rating			Variety mean
	Winnipeg	Morden	Average	
<i>Thatcher</i>				
1	17.4	8.1	12.8	16.6
2	24.0	11.0	17.5	
3	26.2	12.8	19.5	
<i>Renown</i>				
1	23.2	11.4	17.3	21.2
2	26.8	15.7	21.2	
3	31.0	20.4	25.2	
<i>Regent</i>				
1	25.6	11.1	18.4	22.9
2	27.2	16.0	21.6	
3	34.2	23.1	28.6	
<i>Red Bobs</i>				
1	23.4	19.0	21.2	23.1
2	23.5	23.2	23.4	
3	27.0	21.3	24.6	
Necessary difference, 5 per cent level	4.7	7.2	5.0	6.0
<i>All varieties</i>				
1	22.6	12.4	17.5	
2	25.4	16.5	20.9	
3	29.9	19.4	24.6	
Necessary difference, 5 per cent level	2.4	3.6	2.5	

rot in the varieties Thatcher, Regent, and Renown, but only slightly affected root-rot development in the variety Red Bobs. The differences observed in root-rot rating between shallow (1 in.) and deep (3 in.) seeding of Red Bobs wheat were small and statistically insignificant. It is evident, therefore, that the relation between depth of seeding and the incidence of root rot may be modified considerably by the variety grown.

The varieties of wheat used in the experiment of 1944 ranked in order of resistance to root rot as follows: Thatcher, Renown, Regent, and Red Bobs. This ranking is in agreement with that obtained in earlier studies on the relative resistance of wheat varieties to common root rot (9). Thatcher was much more resistant to root rot than was any of the other varieties tested.

DISCUSSION

There is abundant evidence to indicate that in Manitoba the prevalence of common root rot of wheat varies considerably from season to season, and, in any one season, from district to district, and even from field to field. Local variation in the incidence of the disease suggests the possibility of control through crop and soil management. From the results presented in this paper it is evident that variation in the time, rate, and depth of seeding also influences the development of root rot. Early, thin, and shallow seeding reduced the intensity of the disease. This method of control appeared to be effective, and, in the case of common root rot of wheat, offers distinct practical possibilities.

There can be no doubt that there is a close interrelation between soil conditions and crop conditions, and between these and the amount of root rot that develops. The tendency for root rot to be more prevalent in wheat crops that are sown late, too deeply, or too thickly, arises as a result of the interaction of a number of factors. Soil temperature, moisture content, aeration, reaction, and soil nutrients are factors that play a part in modifying the relation between date, depth, and rate of seeding and the development of root rot. Furthermore, these factors are capable of affecting root-rot development not only directly through their effects upon the causal fungi, and upon host resistance, but also indirectly through their effects upon the complex microbiological flora of the soil. The important part different soil conditions play in the development and control of root diseases of wheat and other field crops has been adequately discussed by Garrett (7).

The opinion is widely held in Manitoba that dry seasons are usually seasons of heavy root-rot infection, and this opinion is supported by numerous observations made over a long period of time. For instance, low soil moisture conditions at time of seeding favor infection by retarding the germination of the seed, and the early growth of the wheat seedlings. Again, very low soil moisture content during the post-seedling stages of growth may injure the roots of the plants to such an extent that the entry of parasitic fungi is appreciably facilitated. On the other hand, high soil moisture at time of seeding followed by low soil moisture in the post-seedling stages of plant growth, favors root-rot development. As has been widely recognized, weakened plant growth caused by low moisture conditions constitutes a very important factor in the progress of the disease. The present field studies have indicated, however, that, in Manitoba, early seeding of wheat when the moisture content of the soil is relatively high promotes rapid and vigorous plant growth, and is an effective means of reducing the losses from seedling blight and root rot caused by *Helminthosporium sativum* and *Fusarium spp.* They have also emphasized the need of further field studies to elucidate the many factors responsible for variation in the incidence of root rot in relation to date, depth, and rate of seeding under local conditions.

SUMMARY

The results of field experiments, conducted in Manitoba in 1936, 1937, 1938, and 1939, on the relation between date of seeding of spring wheat and the incidence of root rot (*Helminthosporium sativum* and *Fusarium spp.*) indicated that early seeding reduced the incidence of the disease and increased yield. Soil temperature, as related to date of seeding, was positively associated with root-rot infection and negatively related to yield. Thus, low soil temperature (early seeding) was associated with low disease incidence and with high yield. The effect of high soil temperature on the incidence of root rot, with the resulting effect of the disease on the plants, was an important factor in reducing yields in late-sown crops of spring wheat.

The effect of rate of seeding on the incidence of root rot was studied in 1938, 1939, and 1940. A close relationship was found between rate of seeding and the development of root rot. Each year the severity of the disease increased with thickness of planting.

A close association was found between depth of seeding and the development of root rot. In experiments carried out in 1943 and 1944 root-rot severity increased with depth of planting. As a factor in the control of root rot, depth of seeding was more important in some varieties than in others.

Field experiments have indicated that agricultural practices which favor the rapid, vigorous growth of a wheat crop tend to reduce the intensity of root-rot infection. They have also demonstrated the practicability of reducing losses from *Helminthosporium-Fusarium* root rot in Manitoba by seeding spring wheat not too thickly or deeply, and at the earliest feasible date.

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EXPLORATORY EXPERIMENTS WITH THE BIG-VEIN DISEASE OF LETTUCE

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In 1934, Jagger and Chandler (1) predicted that big vein might become troublesome in lettuce grown on the heavier soils of California and Arizona. At that time the disease was known to be present only in those western states. However, big vein has now been reported from several states along the Atlantic Seaboard (2, 4). It is not known whether this malady was recently introduced into this region, or whether it was merely differentiated from other lettuce diseases after Jagger and Chandler published their report. Since 1934, big vein has not increased particularly in the Imperial Valley, California, or in the Arizona section, but has become more prevalent in the Salinas-Watsonville district. The increased importance of the disease made it seem desirable to investigate further its nature and behavior. Consequently in 1939 several lines of investigation on the disease were initiated with emphasis upon those factors that influence its behavior in trials for disease resistance. Because of the war most of the work on big vein was suspended in 1942, but it seems desirable to publish the results obtained thus far. Although the data in many instances admittedly are only suggestive, they may be of some aid to workers who study the disease.

TRANSMISSION EXPERIMENTS

Insect Transmission

In February, 1941, several flats of lettuce plants from breeding lines were grown from seed in a greenhouse being used for big-vein experiments. The flats were placed on a bench filled with sterilized soil. These plants were transplanted to a disease-free field. Big vein appeared shortly after transplanting and, on June 2, 12.6 per cent of about 2,000 plants were diseased. Since it seemed that insect transmission in the greenhouse may have been the means of infection, attempts were made to transmit the disease with the aphids *Macrosiphum solanifolii* Ashm., *Myzus convolvuli* Kalt., and *M. persicae* Sulz. These species are present on lettuce from time to time in greenhouses at La Jolla. In 18 experiments, with various methods of transfer and different feeding periods, big-vein symptoms occurred in only a very few plants. Consistent transmission even to a low percentage of plants could not be obtained.

Thompson, Doolittle, and Smith (5) obtained very similar results with certain leaf-feeding aphids. On the other hand, their preliminary experi-

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ments with the root aphid *Pemphigus lactucae* (Fitch) indicate that this insect may be an efficient vector. Since root aphids have not been found in the greenhouses at La Jolla, it would seem that other vectors of big vein might exist.

Mechanical Transmission

In 10 trials, involving about 700 plants including checks, no consistent transmission was obtained with leaf juice by (1) leaf-rubbing methods using materials such as cotton, cheesecloth, and carborundum; (2) leaf-punctures using needles through drops of juice from diseased plants and hypodermic needles thrust into the larger veins and into the stem; or by (3) root inoculation by hypodermic needles, or soaking roots in leaf juice from big-vein-infected lettuce.

Seed Transmission

Plants grown on disease-free soil from seed soaked for 24 hours in juice from big-vein-infected plants did not contract the disease.

In one lot of seed from infected lettuce, 18 of approximately 1,000 plants developed big vein. Other extensive trials, however, gave no indication of seed transmission. In view of these results, it appears that big vein is rarely if ever, seed-transmitted.

Soil Transmission

By Soil Extracts. To 3½ kg. of highly infective soil was added 8 liters of tap water. The mixture was thoroughly stirred and then allowed to settle. The apparently clear supernatant liquid was siphoned off, care being taken to collect no macroscopically visible soil particles. This liquid was divided into two parts, one of which was filtered through a medium-grade Mandler filter. The filtered and unfiltered solutions were each divided into five equal parts and added respectively to two groups of five pots of disease-free soil in which small lettuce plants were growing. As a control a similar lot of soil was autoclaved for 2 hours at 15 pounds' pressure, then used like the infective soil to prepare unfiltered and filtered solutions for pot application. In each case the leached soil was also planted to lettuce.

After about 2 months many big-vein plants occurred on the infective, nonautoclaved, leached soil and none on the autoclaved, leached soil. Both of the leachates from autoclaved soil and the filtered leachate from infective soil produced no disease when added to big-vein-free soil (two experiments). However, the unfiltered leachate from infective soil produced four big-vein plants in a total of 83 plants in noninfective soil.

The results corroborate those of Jagger and Chandler (1) in that leaching does not materially decrease soil infectivity. The leaching experiments were too few to warrant definite conclusions, but they seem to indicate either that some infective soil was carried in the unfiltered extract or that the infective particles are nonfiltrable, or that they are absorbed by bodies in the soil that are macroscopically invisible but large enough to be removed by a medium Mandler filter.

By Contamination of Soil with Lettuce Leaves Having Big-Vein Symptoms. In the various experiments, big-vein plants appeared occasionally on soil supposedly not infective. They were cut off at the soil level at the conclusion of the experiment and the pot replanted with lettuce started in disease-free soil. Almost invariably replants developed big vein. To check further on the possibility that diseased plant remains might contaminate noninfective soil, two experiments were run. In the first trial, 650 gm. of coarsely chopped big-vein leaves were mixed with enough soil to fill 15 6-inch pots; in the second, 1,000 gm. were used for 10 pots. The same number of control pots received equal treatments except that healthy lettuce leaves were used. Four plants from disease-free soil were then transplanted to each pot.

All of the check plants remained healthy in both experiments. After about 2½ months, five out of 48 plants surviving in soil mixed with diseased leaves in the first experiment showed big vein; in the second experiment five out of 40 plants had the disease. These results suggest that the infective principle causing big vein can be transmitted to soil by diseased leaves.

EXPERIMENTS ON THE BEHAVIOR OF THE INFECTIVE PRINCIPLE IN THE SOIL

Direct Seeding versus Transplanting in Big-Vein-Infective Soil

In trials for resistance to big vein and in other experiments on infective soil, it is necessary to obtain as high a degree of infection as possible. For this reason, it was desired to learn whether direct seeding in or transplanting to infective soil produced the greatest number of diseased plants. Three trials involving a total of 112 plants from seed and the same number from transplants indicated a very slight tendency for more big vein to develop in the transplants. Since transplants are generally used in the big-vein susceptibility tests at this station, this line of investigation was dropped.

Spread Through the Soil from Diseased Plants

To discover whether big vein would spread from a diseased plant to adjacent healthy plants, one small big-vein plant and three healthy plants were set near the rim and 90° apart in each of eight 6-inch pots, in each of three experiments. The plants were grown for 2 to 2½ months. All of the big-vein plants showed the disease throughout the length of each run. In the first trial, two of the 24 healthy plants developed big vein; in the second, one doubtfully diseased plant occurred; and in the third, the originally healthy plants remained so during the course of the experiment.

It is possible that the disease development in these experiments might have resulted from insect transmission. In any event, it appears that big vein spreads very slowly, if at all, through undisturbed soil.

Effect of Drainage on the Development of Big Vein

In earlier studies on soil moisture in relation to big vein (3) it was noted that in soil from the Imperial Valley, California, salt concentrations built

up to such a point that plant growth was reduced. In some experiments not reported in the earlier paper there was a rather pronounced decrease in infectivity as the salt concentration increased. An analysis² was made of two lots of soil identical except that salt had been built up in one through continued use in pots without drainage and that this high-salt soil was considerably less infective. The total salt concentration in the highly infective soil was less than $\frac{1}{2}$ that in the soil of low infectivity (Table 1). The very great difference in amount of sulphate in the two lots of soil is notable.

Since the increase in salt content and decrease in infectivity apparently resulted from use of pots without drainage, four experiments were run in which the development of big-vein plants in drained and nondrained pots was recorded. In general there was a slight tendency for fewer diseased plants to appear in the nondrained pots than in the drained but in only one trial was this difference statistically significant and the mean difference of

TABLE 1.—*Analysis of two lots of soil differing in big-vein infectivity and in salt concentration*

Soil infectivity	Parts per million in soil extract								
	Na	Pb	Kc	Ca	Mg	Fe	SO ₄	Cl	Total
Low	106	15	62	200	Tr.	15	500	50	948
High	54	4	60	150	Tr.	13	Tr.	10	291

^a Includes NO₃, NH₄, NO₂.

^b Includes reserve P.

^c Includes reserve K.

all tests was nonsignificant. Only one crop of lettuce was grown in each pot. It may be that had two or more crops been grown in each pot the differences would have become more pronounced.

Time Required for Infection in Big-Vein-Infective Soil

Lettuce seed was sown in highly infective big-vein soil. Two weeks after planting the lettuce was up but only the cotyledons had unfolded. Twenty plants were removed at this time, washed thoroughly in tap water and transplanted to disease-free soil in 6-inch pots with four plants per pot. At weekly intervals thereafter, an additional 20 plants were removed, washed, and transplanted. When big vein appeared in lettuce on the big-vein soil, transplanting was discontinued.

The amount of big vein occurring in the transplants about 10 to 16 weeks after seeding is shown in table 2. This type of experiment is open to the criticism that it is impossible to be sure that all of the infective soil was removed in washing. Furthermore, since nothing is known about where infection takes place in the root system or how fast the causal entity moves after infection there is the possibility that the infected portion of the root systems may have been separated from healthy portions in the transplanting

² The author is indebted to John H. Pryor, Growers Ice and Development Company, Salinas, California, for making this analysis.

process. Nevertheless, it is apparent that some plants did not become diseased within 6 weeks from seeding while others seem to have been infected almost as soon as they emerged from the seed. Earlier observations (3) indicated that big vein develops best in vigorously growing plants. Since transplanting undoubtedly slows down the growth rate for a time it might be expected that when those plants with the longer time intervals in infective soil were held in disease-free soil for equivalent lengths of time, a greater proportion of big vein would develop in the later transplants. The last two columns of table 2 show this to be true. From this work it may be tentatively concluded that at least four weeks is required in big-vein soil for a large proportion of the plants to become infected.

Effect of Soil Dilution on Infectivity

In the first of these experiments, dilutions of infective big-vein soil with autoclaved big-vein soil did not reduce disease at the maximum dilution of

TABLE 2.—*The amount of big vein appearing in lettuce after transplanting seedlings grown from seed sown in infective soil**

No. weeks in infective soil	Counts after variable time in disease- free soil				Number of big-vein plants after 10 weeks in disease free soil	
	No. weeks in disease-free soil	Number of big-vein plants				
		Test 1	Test 2	Test 3	Test 1	Test 2
2	8	2	4	2	2	4
3	7	3	4	11	3	5
4	6	6	11	11	6	15
5	5	6	9	15	8	14
6	4	3	14		6	18

* Twenty plants grown at each exposure in each test.

1 part infective soil to 32 parts of sterilized soil. In another experiment the maximum dilution of 1 part to 800 reduced disease only slightly. The autoclaved soil in each case had only healthy plants. It is unwise to generalize from such few data, but this high dilution with little reduction in disease may indicate that the soil on farm implements might be effective in spreading the disease.

Persistence of Big-Vein Infectiveness in Soil

In 1936 a lot of big-vein soil was collected in the Imperial Valley of California and stored in a large, covered, galvanized-iron can in a dry place. From 1940 to 1944 small portions of this soil were removed periodically, tested for infectivity, and then discarded. Parallel checks were not run; however, checks in other experiments seldom became diseased and when they did the amount was never greater than 1 or 2 per cent. It is evident that big vein will persist with no decrease in infectivity for at least eight years in stored, air-dry soil (Table 3).

TABLE 3.—*Persistence of big-vein infectiveness in stored, air-dry soil*

No. years in storage	4	5	7	8
No. plants tested	33	24	6	24
Percentage of plants with big vein	33	54	83	75

*The Influence of Soil Temperatures on the Production of
Big-Vein Symptoms*

Methods. Five "Wisconsin-type" soil-temperature tanks each containing eight 6½ × 9-inch round galvanized-iron culture cans were used for the temperature studies. The tanks were located outdoors and a movable shelter was employed to cover them when there was probability of rain. Temperature was controlled by hot or cold water introduced through a perforated pipe running lengthwise and parallel to the bottom of the tank and about 1½ inches below the bottoms of the cans. When the tanks were operated below air temperatures, the water was cooled by a small refrigeration unit. The addition of hot or cold water was controlled by solenoid valves actuated by poultry-incubator-type thermostats³ which were sensitive to about ± 0.5° C. Temperatures used were 14°, 18°, 22°, 26°, and 30° C.

The infective soils were the ones employed in the moisture studies (3). The soil line in the cans was slightly below the water line in the temperature

TABLE 4. *Effect of soil temperature on the development of big-vein symptoms in lettuce*

Experiment No. ^a	Time of the experiment	Percentage of big vein plants at various soil temperatures (32 plants initially at each temperature in each experiment)					Total percentage of big-vein plants	Average daily air temperature ^b		
		14° C.	18° C.	22° C.	26° C.	30° C.		Maxi- mum °C.	Mini- mum °C.	Mean °C.
1	Dec. 31, 1941, to Feb. 18, 1942	7	22	32	34	17	23			
2	Mar. 5 to May 1, 1942	38	48	60	48	10	41	18.1	8.5	13.3
3	Nov. 6, 1942, to Jan. 11, 1943	88	90	93	68	38	78	18.9	7.6	13.3
4	Feb. 26 to May 15, 1943	87	93	96	22	5	64	18.2	10.5	14.3
5	July 20 to Sept. 14, 1943	88	97	75	16	0	56	23.6	16.2	19.9
6	Nov. 22, 1943, to Feb. 19, 1944	84	90	94	54	10	67	17.2	6.8	12.1
7	Apr. 5 to June 13, 1944	94	84	97	34	21	67	18.6	11.3	14.9

^a Experiments one through five were with Salinas soil; six and seven were with Imperial Valley soil.

^b The mean temperature is the average of the daily extremes. No reliable air temperature records were available for experiment number one. The refrigeration unit was out of order from May 2, 1944, to June 13, 1944, when experiment 7 was run.

³ Type SA9-3Z obtained from Lyon Rural Electric Company, San Diego, California.

tanks and 1½ inches below the rims of the cans. Four lettuce plants with one or two true leaves were transplanted to each can after the soil had reached the desired temperature. The top of the soil was insulated with 30 gm. of rock wool. Enough water was added at short intervals through a central perforated pipe in each can to maintain the moisture at 75 per cent of the moisture-holding capacity of the soil (3). Plants were examined for big-vein symptoms at each watering. When there was evidence of crowding or head formation the experiment was discontinued.

Results. The data from several experiments are in table 4 together with the air temperatures prevailing during the trials. It is apparent that, with the exception of experiments number one and five, the greatest percentage of disease developed was at 22° C. There was generally a sharp decrease in the amount of big vein at 26° and 30° C. and some decrease at 14° and 18° C.

From reports in the literature (1, 4) air temperature also seems to have an influence on the production of big-vein symptoms and might have caused the discrepancy mentioned above. With this possibility in mind, air temperature records had been kept during most of the trials. No adequate records were available during the course of the first experiment, but from those at hand it appeared that the minimum air temperature at least was somewhat lower than in the other trials. Observations also indicated that the infectivity of the soil in the first two experiments was not so high as in the later ones. With these facts in mind, the apparent suppression of symptoms at higher soil temperatures in the fifth experiment might be explained as an effect of the substantially higher air temperature. Comparing the air temperatures with the amount of big vein at different soil temperatures, it is evident that concurrently with the higher air temperatures during July, August, and September, in experiment number 5, the optimum soil temperature was lowered to around 18° C. There is also a slight indication that the higher optimum soil temperature during the first experiment might have resulted from lower air temperature.

In each of the temperature experiments the initial number of lettuce transplants was 160, or 32 for each treatment. In some cases the survival was not very satisfactory, particularly at the higher soil temperatures (26° and 30° C.). This is not surprising since lettuce is known to be a low-temperature crop.

Discussion. Jagger and Chandler (1) and Thompson and Doolittle (4) observed that low temperature favors the development of big-vein symptoms. Since big vein is a soil-borne disease, it was thought that soil temperature might be more important than air temperature in producing this effect. While preliminary results reported here indicate that big vein will develop in lettuce plants grown at a constant soil temperature of 30° C., Thompson and Doolittle (4) observed that greenhouse air temperatures of 65° to 75° F. during the day and 50° to 60° F. at night prevented the appearance of big-vein symptoms in lettuce leaves. When the temperature was lowered to 50° to 60° F. during the day and to 45° to 50° F. at night they found that big

vein developed in some plants. Greenhouse temperatures again raised to the higher level suppressed symptoms. The fifth experiment reported in the present paper was carried out when air temperatures were comparable to those employed by Thompson and Doolittle (4) yet some big vein was evident at a soil temperature of 26° C. (78.8° F.), considerable was present at 22° C. (71.6° F.), and still more occurred at lower soil temperatures. During the course of this experiment, the range of maximum air temperature was 70° to 83° F. and the range of the minimum was 55° to 68° F. The discrepancy in results obtained in the two investigations possibly may have been due to the maintenance of a longer period of high air temperature in the greenhouse than occurred in the present work. Other explanations may be forthcoming with further research.

Indications are that when air temperature is favorable for the production of big-vein symptoms a soil temperature of about 22° C. is optimum for disease development in the leaves. However, considerably more work is necessary before the effect of air temperature and the combined effects of soil and air temperatures can be properly evaluated.

SUMMARY

Experiments using three species of leaf-feeding aphids, various methods of mechanical inoculation, lettuce seed soaked in leaf juice from diseased plants, and seed harvested from diseased plants resulted in no consistent transmission of big vein. The unfiltered leachate from infective soil when added to disease-free soil produced big vein in four of 83 plants grown thereon. The filtered leachate (medium Mandler filter) caused no disease. Big vein was not eliminated from infective soil by leaching. Symptoms appeared in plants grown on big-vein-free soil to which chopped diseased leaves were added. None appeared in comparable checks.

There was little difference in the amount of big vein developing in transplants and in lettuce seeded directly in infective soil. Trials in which one big-vein plant was grown for 2 to 2½ months adjacent to three healthy plants in a 6-inch pot filled with disease-free soil indicated that big vein spreads very slowly if at all through the undisturbed soil during the period used. A slight tendency existed for fewer diseased plants to appear in undrained pots filled with Imperial Valley soil than in drained pots but in only one experiment was this difference statistically significant. Transplanting lettuce at intervals from infective to noninfective soil indicated that some big-vein infection takes place within two weeks from seeding; under the conditions imposed, at least four weeks' growth in big-vein-infective soil was required for a large portion of the plants to become infected. Exploratory trials indicated that diluting 1 part of big-vein soil with 800 parts of autoclaved soil reduced disease incidence only slightly. Data obtained thus far show that stored, air-dry soil remains infective for at least eight years. When air temperature was favorable, the optimum soil temperature for development of big vein appeared to be about 22° C. (71.6° F.), with some

big vein occurring at all soil temperatures from 14° to 30° C. However, air temperature also seemed to have some effect on symptom expression.

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PEACH BLOTCH¹

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In the course of an intensive survey of peach orchards in a two-square-mile block near Winona, Ontario, conducted in the years 1937 to 1940 in connection with some studies of peach yellows, mosaic-like variegations were observed in 1940 on a single three- or four-year-old tree of an unknown large-flowered variety. The variegations were well-defined pale green to yellowish green areas very variable in size and shape, ranging from numerous angular spots scattered over the leaf surface (Fig. 1, A) to larger, irregular blotches usually fewer in number (Fig. 1, B, C). A few leaves were marked only by a yellowing along the larger veins (Fig. 1, H). Affected leaves as a rule were not distorted though some had ragged margins (Fig. 1, D), resulting from marginal scorch. The fruit was not affected and no marking or stunting of twigs was observed. Peach blotch has been adopted as the name for this disorder, as being characteristic of the predominating symptom.

Preliminary inoculations of peach seedlings by budding, in 1940, demonstrated the graft-transmissible nature of the variegation, symptoms appearing on the seedlings the following spring. In 1942, transmission tests were extended to include several peach, plum, and cherry varieties which, except for one seedling cherry, were inoculated by the double-budding technique (5). In these tests, peach, Myrobalan, and Mahaleb seedlings were used for stock.

Peach seedlings varied considerably in their response to blotch with respect both to symptom expression and to susceptibility. A few were resistant, showing only slight symptoms on early leaves, but the majority were more or less susceptible, symptoms being found on leaves of all ages. The markings were, in scope and variety, typical of those observed on the original tree, with a few additional variants. One of the latter was an almost symmetrical chlorotic blotch centered about and extending along the mid-rib and tending to feather out along the lateral veins (Fig. 1, F). Inversions of this pattern also occurred (Fig. 1, G) in which the chlorosis was peripheral. Occasionally leaves were completely chlorotic, except for a few, scattered, darker green islands (Fig. 1, E), an inversion of the angular spot pattern found on the original tree. Usually most patterns could be found on a single seedling though sometimes one type of pattern tended to predominate. The amount of scorch varied from seedling to seedling and from season to season. Though large necrotic spots were sometimes to be found, necrosis was typically marginal.

Of the peach varieties tested, Elberta and Rochester were most susceptible. The former displayed symptoms much like those on the original tree,

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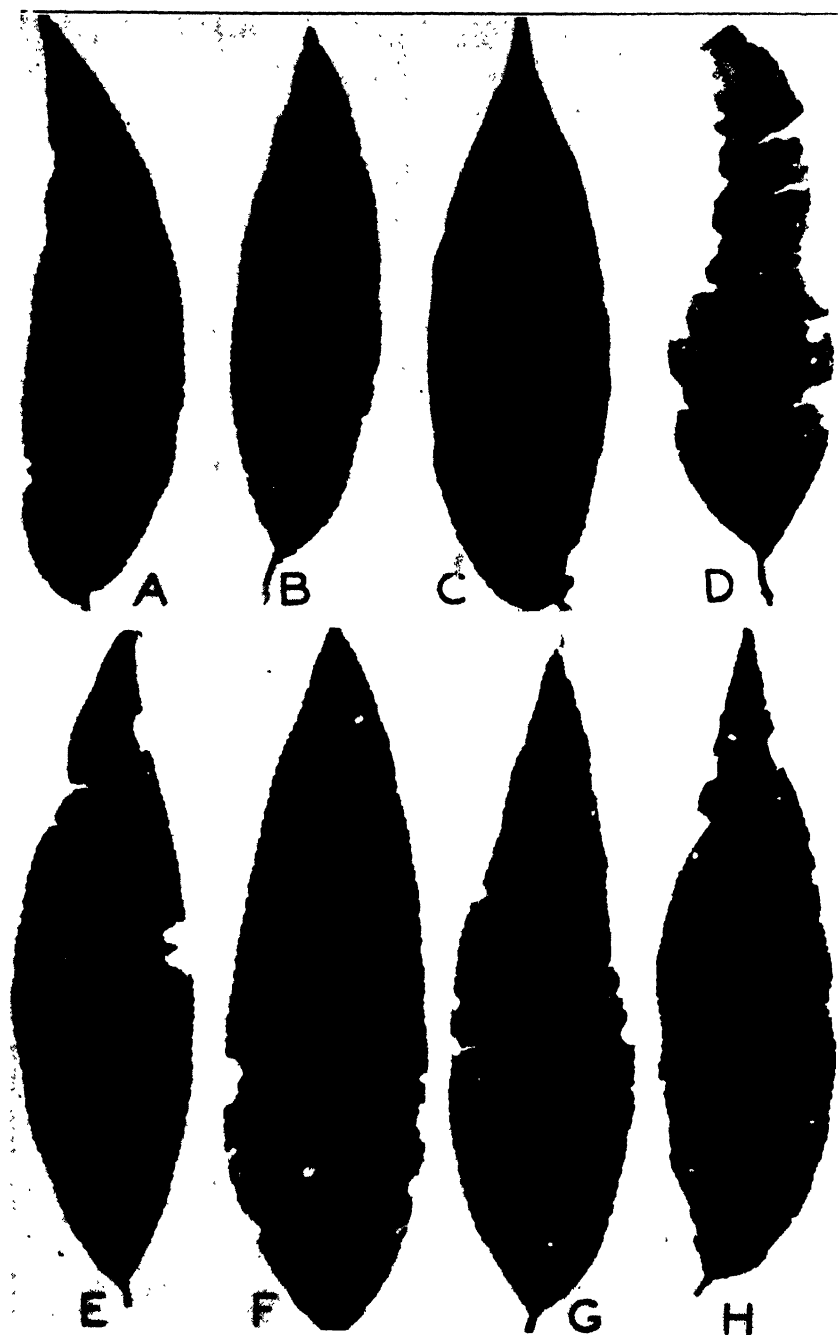


FIG. 1. Pattern variations of peach blotch on peach leaves. A. Small, angular, chlorotic spots. B, C. Irregular blotch patterns. D. Peripheral chlorosis and emargination by scorch. E. Inversion of A. F. Central feathery chlorosis. G. Peripheral chlorosis, inversion of F. H. Faint general chlorosis along veins. A, B, H, from original tree; C, D, E, F, G, from inoculated seedlings.

whereas in the latter, the feathery pattern (Fig. 1, F) predominated. Vedette showed slight blotching in some seasons and little or none in others. Golden Jubilee was almost symptomless, though a few early leaves sometimes had either a mild mosaic pattern or yellowing of veins. Few if any symptoms were observed on Peregrine, a white-fleshed variety imported from England. On all peaches, the patterns were either pale green, yellowish green, or greenish yellow, but never white, and no indications of symptoms were seen on flowers or twigs.

To date, transmission experiments with plum, apricot, and cherry varieties and the cherry seedling have given negative results in at least two seasons since inoculation except for a vague and doubtful blotching on Italian prune. In most cases, the inserted diseased peach buds grew, and even where they did not, union with the stock took place so that transmission of the virus to each of the test varieties was possible. The varieties used in these experiments included: *Prunus domestica* var. Italian prune, German prune, Reine Claude, and Lombard; *P. salicina* var. Abundance; *P. cerasifera*, Myrobalan seedlings; *P. armeniaca* var. Niagara; *P. avium* var. Black Tartarian, Napoleon, and seedling; *P. cerasus* var. Montmorency, and *P. mahaleb* seedlings. The double-budding tests, however, should be supplemented by inoculation of the same varieties after they have been indexed and established as nursery stock. Whether or not plums and cherries can act as carriers of blotch has not yet been determined.

In 1944, what appeared to be a second natural occurrence of blotch was found on sucker growth near the crotch of a tree of the Marigold variety in an orchard near Hamilton, Ontario. In this case, the predominating symptom type was the central, symmetrical, feathery blotch or some modification thereof. Inoculation of peach seedlings has demonstrated transmissibility, but the effects of this virus on other hosts are yet to be worked out.

Two other infectious variegations of the peach, mottle and calico, have been investigated by Blodgett (1, 2). Reeves (4) has also found isolated cases of a calico-like disorder on peach in Washington. Blodgett's descriptions of mottle and calico and his illustrations of calico indicate that these two diseases bear considerable resemblance to peach blotch, both in their foliage symptoms and in the rarity of their occurrence in nature. On the other hand, these three diseases and presumably their causal viruses are not identical. For example, the peach mottle virus (1) causes not only severe mottling, deformity and necrosis of leaves, and dwarfing of trees of sweet cherry but also die-back and gummosis of Montmorency, whereas the blotch virus and the calico virus (2) have had no apparent effect on cherry varieties. Calico also differs from blotch, though less markedly, in that its patterns become "brilliant-yellow, almost papery-white" and appear on twigs and fruits as well as on leaves. Furthermore, among the symptoms of calico no mention is made of the marginal lacerations and scorch associated with both blotch and mottle.

As these three peach disorders are not of great economic importance at

the present time, their chief claim to interest lies in the possible implications of the characteristics they have both individually and in common, considered in relation to their distribution. Their foliage symptoms on peach suggest that the diseases may be caused by virus strains, more or less closely related but differing in virulence. The differences between the diseases could then be construed as differences in degree rather than in kind. It is difficult, however, to see how such strains could arise when the disorders they produce occur as virtually isolated cases in widely separated districts. It is, of course, possible that the diseases may be sporadic infections by pre-existent viruses not ordinarily transmitted to peach. In such an event there would seem to be undue stretching of coincidence to expect sporadic infections of that type, even under necessarily highly exceptional conditions, to be confined to single trees in an orchard or district, particularly if more than one virus is involved. Blodgett's suggestions (2) that some viruses "may be originating within rather than being contracted by certain host plants" and that "the differences between perpetuated abnormalities and transmissible diseases, such as calico, may be, after all, fundamentally rather slight" deserve some consideration, especially in the light of recent work by Woods and Du Buy (3, 6). These investigations indicate the possibility that infectious as well as noninfectious variegations may arise through alterations in the chondriosomes or plastids. Such an explanation of the origin of the diseases under discussion could account both for their peculiar distribution as well as for their points of similarity and dissimilarity. However, until more than circumstantial evidence is forthcoming, the relationships between the causal viruses themselves and between the viruses and their hosts must remain enigmatic.

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GROWTH AND OVERWINTERING OF XANTHOMONAS VESICATORIA IN ASSOCIATION WITH WHEAT ROOTS¹

STEPHEN DIACHUN AND W. D. VALLEAU

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In previous reports it has been demonstrated that the tobacco leaf-spot bacteria, *Bacterium tabacum* and *B. angularum*, can form colonies on roots of wheat and other unrelated plants, and, furthermore, that these two organisms can maintain themselves in field soil indefinitely, apparently in association with roots (6, 7).

The investigation has been extended to find out, if possible, whether plant pathogens other than the tobacco leaf-spot bacteria can perpetuate themselves in association with roots. The selection of species to be tested is somewhat limited by the method used in the study. To determine the presence or absence of pathogenic bacteria in soil or on roots, an aqueous suspension of the suspected soil or roots is poured on an artificially water-soaked leaf of the appropriate host. The leaf is water-soaked by squirting a stream of water from a hypodermic syringe against the lower surface; water-soaking occurs only if the stomata are open, and remains only a few minutes. Water-soaking, if properly done, does not seem to injure leaf cells, as indicated by the fact that when tobacco-mosaic virus is poured over water-soaked leaves of mosaic-resistant plants (NN) no necrotic spots result (2). If a suspension of bacteria is placed on such a temporarily water-soaked leaf some of the bacteria enter the leaf, in the water-soaked area, and, if pathogenic, they subsequently produce leaf spots (1); but temporary water-soaking does not increase susceptibility to nonpathogenic organisms. Thus, development of spots on leaves inoculated in this way with suspected roots or soil shows that pathogenic bacteria were present in the tested material, and failure of spotting indicates absence of the bacteria, or perhaps presence in very small numbers.

Xanthomonas vesicatoria, *X. phaseoli* var. *sojense*, and *Bacterium medicaginis* var. *phaseolicola* were selected for study. They cause common, widely distributed diseases, and appear to differ somewhat among themselves in the method of overwintering. Epidemics of halo blight of beans, caused by *B. medicaginis* var. *phaseolicola* are generally believed to be initiated largely by seed-borne organisms (4). Also, much of the primary infection caused by *X. vesicatoria*, is thought to be traceable to contaminated seed, but because epidemics have occurred in plants grown from seed believed to be clean, there is reason to believe that primary infection can originate in some other unknown source, perhaps contaminated soil (3). Primary inoculum for outbreaks of soybean bacterial pustule, caused by *X. phaseoli*

¹ The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

var. *sojense*, is commonly thought to come from overwintered organisms in fallen diseased leaves (5).

In the present report are given the results of tests designed to determine whether these 3 organisms can grow on roots and also overwinter in soil in association with wheat roots.

Colony Formation on Roots. The ability of these species of bacteria to grow on roots was tested on 4 species of plants. Seeds of Thorne wheat, Rutgers tomato, Stringless green-pod bean, and Macoupin soybean were surface-sterilized by soaking 2 hours in a 1.25 per cent solution of NaOCl, then germinated on sterile moist paper towel in sterile Petri dishes at 27° C. in the dark. In 2 days roots of the wheat seedlings were large enough to be inoculated (soybean, 2 days; bean, 3 days; tomato, 4 days). Inoculum was prepared from 48-hour cultures on slants of potato-dextrose agar (0.5 per cent dextrose). The bacteria were suspended in sterile tap water, 1 to 2 million cells per cc. In each test, roots of 10 plants were immersed in the

TABLE 1.—Colony formation by 3 species of plant-pathogenic bacteria on roots of seedlings grown under aseptic conditions

Bacterial inoculum	Roots* on which bacteria developed			
	Wheat	Tomato	Bean	Soybean
<i>Xanthomonas vesicatoria</i>	38/40	11/30	7/10	6/10
<i>Bact. medicaginis</i> var. <i>phasicicola</i>	20/30	2/20	19/20	6/10
<i>X. phaseoli</i> var. <i>sojense</i>	29/30	4/20	8/10	5/10
No inoculation	1/110	0/50	0/30	2/30

* The denominator of the fraction shows the total number of plants of which the roots were inoculated. Since 10 plants were used in each test this figure also gives indirectly the number of tests. The numerator is the number of plants on the roots of which bacterial colonies were present.

inoculum. The inoculated plants were then so placed on sterile moist paper towels in sterile Petri dishes, 3 or 4 plants per dish, that the roots were in the moist air, not in contact with paper or glass. Two days later examination of the roots (mounted in water) with the low power of a microscope showed whether colonies were present. Some of the tests were repeated several times, as shown in table 1.

In all the tests all 3 species of bacteria multiplied and produced colonies on the surface of the roots of wheat. Roots of bean, soybean, and tomato seedlings also supported growth of the bacteria but perhaps not so consistently as wheat (Table 1). In general, there seemed to be no tendency for the bacteria to grow better on the roots of their usual host plants than on the roots of at least one other plant tested. In fact wheat roots seemed to support growth of *Xanthomonas vesicatoria* and *X. phaseoli* var. *sojense* better than did tomato and soybean.

Overwintering in Association with Wheat Roots. The ability of these species of bacteria to overwinter in soil in association with wheat roots was tested. On November 11, 1943, Thorne wheat was sown outdoors in each of 3 small plots, 3 × 5 feet, of spaded nonsterilized soil. Immediately, 750 cc.

of a 6-day beef-peptone-broth culture of one of the 3 species of bacteria was sprinkled on each plot. Each plot was then raked separately with a sterile rake, and sprinkled with 10 liters of tap water. At about monthly intervals between December 11, 1943, and April 14, 1944, ten plants were dug from each plot. The soil was washed from the roots in running tap water. The root system of each plant was crushed separately in a sterile aluminum dish with a sterile glass spatula with 10 cc. tap water, and poured on the lower surface of a water-soaked leaf of the appropriate host: *i.e.*, tomato, soybean, and bean. Development of leaf spots on the inoculated leaves was regarded as evidence of the presence of bacteria on or near the wheat roots. As a further precaution in each test bacteria were isolated from the spots and compared with colonies from the original culture.

Xanthomonas vesicatoria was recovered from wheat roots in December, January, February, and March, but not in April (Table 2). In each test

TABLE 2.—*Recovery of Xanthomonas vesicatoria from roots of wheat plants grown in inoculated soil outdoors in winter of 1943-44 at Lexington, Kentucky. Root systems of 10 plants crushed individually in water and poured on 10 water-soaked tomato leaves*

Date of isolation	No. spots on inoculated leaves											
Dec. 11, 1943	2,	0,	7,	15,	0,	0,	3,	12,	1,	0		
Jan. 12, 1944	0,	5,	0,	0,	0,	0,	0,	0,	1,	0		
Feb. 1, 1944	0,	0,	0,	1,	1,	0,	8,	0,	0,	0		
Feb. 23, 1944	27,	90,	75,	100,	80,	0,	0,	0,	2,	3		
Mar. 14, 1944	9,	7,	0,	3,	10,	0,	0,	0,	0,	0		
Apr. 14, 1944	0,	0,	0,	0,	0,	0,	0,	0,	0,	0		

X. vesicatoria was isolated from typical spots on the tomato leaves. Also, in the tests of December, February 23, and March, the isolated bacteria were inoculated onto tomato leaves and produced typical spots. Neither *Bacterium medicaginis* var. *phascolicola* nor *X. phascoli* var. *sojense* was recovered from the wheat roots at any time during the period of the test. Although this need not necessarily be interpreted to mean that these organisms cannot overwinter on wheat roots, the general observation that bean halo blight can be avoided by planting disease-free seed may mean that this organism cannot perpetuate itself readily in soil. The tomato and tobacco leaf spots cannot be controlled by planting clean seed; *i.e.*, the organisms exist somewhere else, probably on roots of some nearby plants.

It will be desirable for individual workers to determine the ability of other bacterial pathogens to live and overwinter in association with roots. It would indeed be surprising if the tomato- and tobacco-leaf-spot organisms were unique in this respect. Several or many bacteria may be found to be able to overwinter in association with roots of unrelated species, and perhaps some pathogenic fungi can do likewise—if so, some of the concepts of host range and control of plant diseases by crop rotation may need to be reconsidered.

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FAILURE OF BUD AND GRAFT UNIONS OF ROSE INDUCED BY CHALAROPSIS THIELAVIOIDES

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The "black mold" or "Manetti mold," *Chalaropsis thielavioides* Peyr., has become important in recent years as a cause of graft failure of rose in the east and midwest. The fungus also caused a failure of bud unions in field-grown roses in one area of California during the single year, 1940.

The fungus was perhaps first found on walnut shells from Scotland in 1878 and on apricot roots in Australia in 1902 (5, 9, 11). It was first described (13) as the cause of a root rot of lupine (*Lupinus albus*) in Italy in 1915 and the genus *Chalaropsis* was erected at that time. It was again reported on lupine (*L. albus*) roots in Italy (14) and Germany (12) in 1927. In 1930 it was very damaging to walnut grafts and occurred on carrot roots in England (5). In 1932 the fungus was observed on layered walnuts and on walnut roots in England and it was reported in Palestine in 1933 on peach seedlings from France (5, 9). The fungus was isolated from rose cuttings in Victoria, Australia, by Fish in 1936 (9).

The first definite report of *Chalaropsis thielavioides* in North America seems to have been from Oklahoma in 1934 where it was causing root rot of stored Chinese elm (6). In 1938 the same species caused serious loss of roses in grafting frames in Illinois and New York on rootstocks of *Rosa chinensis* var. *Manetti* from a small area of northern Oregon (8). The disease apparently had been present for some years before discovery (10). In 3 nurseries of Santa Clara County, California, the fungus caused a failure of bud unions on understocks of Manetti, *Rosa odorata*, and *R. multiflora* in 1940 (1); in one field only 26 per cent of the bud inserts had united in 3 successive attempts. It is noteworthy that these losses occurred during the hot, dry months of August and September and that roses budded in a nearby field the following year on cuttings from these infected plants sustained no detectable loss from the disease. In 1945 roses grafted by one grower in Alameda County failed because of decay by this fungus; since it is unusual to graft roses in California, this type of trouble is rare. The disease has not been found in southern California, perhaps because roses there are largely grown on immune Ragged Robin (*Gloire des Rosamanes*) rootstock and are field-budded rather than grafted. The graft disease also has been observed in Pennsylvania and the fungus found on Washington-grown understocks (19). The graft disease apparently has been associated only with American-grown rootstocks.

The fungus is of importance primarily on greenhouse roses in grafting frames and in nurseries where the plants are budded. Because outdoor roses commonly are propagated on the immune Ragged Robin or resistant *Rosa multiflora* rootstocks, little trouble has been reported or would be expected in that crop.

SYMPTOMS

Usually the disease on rose is first detected by the failure of the bud or scion to unite with the stock. On the interfaces of the bud shield or scion and the rootstock frequently is seen a crust or crumbly mass of black spores and this gives rise to the name "black mold." If, however, the specimens are not observed within a few days the cut surfaces frequently are overrun by secondary fungi which may obscure or obliterate macroscopic evidence of the causal organism; macroconidia may still be observed under the microscope at this stage and afford evidence of the disease.

The earliest stages are frequently overlooked because they are less conspicuous than those outlined above. Very quickly after germination of the



FIG. 1. Failure of Peerless buds to unite with stocks of *Rosa chinensis* var. *Manetti*. Inoculated with a suspension of spores of *Chalaropsis thielariioides* after being budded and tied with grafting rubber; held in cutting frame for 24 days before being photographed. Two stems at left are noninoculated checks showing healthy callus formation. Three stems at right show representative bud failures.

infecting spore there is formed a small area of frosty white endoconidia and scant mycelium which spreads rapidly to cover the cut surfaces. This growth is most conspicuous with high humidity and may be very sparse under dry conditions. On newly infected bud shields and wounded *Manetti* stems under humid conditions drops of clear liquid may appear for a short time, drying to leave a shiny spot. The frosty white growth darkens within a few days due to color change of the infected tissues and of the fungus itself, and gradually gives way to the black crust of macrospores.

The bud shield is killed and permeated by the fungus. A scion is killed by being cut off from its moisture supply but may not be completely invaded by the fungus. A susceptible type of rootstock may be invaded for a short distance from the cut, but in the immune Ragged Robin only a surface staining has been noted. The fungus does not attack vigorously growing tissue with the rapidity or degree that it does dormant or senescent wood such as bud shields, scions, cuttings, or branch stubs. Cambial activity of such infected tissues is suppressed and no callus is formed.

The graft phase of the disease is well illustrated elsewhere (3, 8, 10). The symptoms of bud failure seen in California are shown in figure 1.

The fungus attacks the basal end of a cutting, preventing formation of callus or roots and sometimes causing its death. Cuttings less seriously affected may show retarded growth of shoots, perhaps because of impaired water absorption through the callus. Leaves cannot be infected (8); that this is not characteristic of chlorophyllous tissue is shown by the susceptibility of green stems of such varieties as IXL and *Rosa multiflora Grifferaia*. Infection of rose roots is known (8) but apparently causes little damage.

The pronounced fruity odor of the fungus on agar media and on rose can be detected in a closed space such as a grafting case; it is said to be due to isobutyl acetate (8).

THE PATHOGEN

The fungus, *Chalaropsis thielavioides*, which caused this disease of rose in California, is identical in cultural and morphological characters with the fungus reported in New York and Oregon (described and illustrated in 8). Also very similar morphologically, but differing somewhat in cultural characters, are the forms from walnut, lupine, Chinese elm, and peach (described and illustrated in 5, 6, 9, 13). The rose fungus has been reported as similar to the imperfect stages of *Ceratostomella radicola* from date palm (2), *Ceratostomella* sp. from plane trees (17), and *Endoconidiophora variospora* from chestnut oak (4). It was found, however, that the rose fungus did not pair with either strain of *C. radicola* or *E. variospora* to form perithecia.

All workers are agreed that *Chalaropsis thielavioides* is a wound fungus unable to infect uninjured tissue. The published evidence and our findings are in agreement that the fungus only slightly attacks vigorously growing tissue and produces the most severe damage on dormant parts such as stored Chinese elm roots, rose bud shields, graft scions or cuttings, walnut scions, etc.

The fungus invades the xylem, cortex, vascular rays, and pith of rose and walnut stems, forming macroconidia in the vessels (5, 8).

HOST RANGE AND RESISTANCE¹

There have been 6 physiologic forms of *Chalaropsis thielavioides* reported; these have been designated as the lupine (13), walnut (5), carrot (5), peach (5), Chinese elm (6), and rose (10) forms. While these forms

¹ Some of the rootstocks used in this work were kindly supplied by Armstrong Nurseries, Ontario; California Nursery Co., Niles; and Western Rose Co., San Fernando.

have been described as differing slightly in cultural and morphological characters, the prime distinction is physiological, resulting in host specificity. That there is, however, physiological uniformity within the species is shown by the fact that the lupine, elm, and rose forms are consistent in vitamin requirement, lacking ability to synthesize thiamine but able to form biotin and pyridoxine, whereas other species differ in these requirements (15).

Tests were made to determine partial host range of some of these forms and of some closely related fungi. The California and New York² isolates of the rose form of *Chalaropsis thielavioides* strongly parasitized wounded rootstocks of Manetti, *Rosa odorata*, and *R. multiflora*, but did not infect Ragged Robin. The forms of *C. thielavioides* from lupine,³ walnut,⁴ and Chinese elm,⁵ *Ceratostomella radicicola* from date palm,⁶ and *Ceratostomella fimbriata* from sweet potato were unable to parasitize any of the 4 rose rootstocks. *Endoconidiophora* sp. from plane tree⁷ did not infect Manetti rootstock but was slightly parasitic on Ragged Robin. *Endoconidiophora vari-ospora* from chestnut oak⁸ and *Thielaviopsis basicola* from tree peony were nonpathogenic to Manetti and Ragged Robin.

A California isolate of the rose form was tested for pathogenicity on a number of other plants. A freshly peeled area on each stem was inoculated with a pure culture and the stem held in a moist chamber. *Rosa laza*, once used as an understock, was found to be immune, *R. longicuspis*, *R. nutkana*,⁹ and *R. californica*⁸ were moderately susceptible, *R. gymnocarpa*, *R. multiflora* Griffithiae, and the rootstock IXL were very susceptible. Stems of orange and *Chaenomeles japonica*, and roots and stems of *Ulmus pumila* were slightly susceptible, stems of pear,⁸ sweet cherry, peach, almond, Mariana plum, and Persian and black walnut were moderately susceptible, and apple was immune.

Longrée (8) found that the rose fungus grew slightly on wood of Chinese elm, black walnut, English walnut, poplar, and peach, and on potato tuber and carrot roots in moist chambers. Some growth of the elm form was obtained on wounded rose stems under the same conditions. Hamond (5) reported successful inoculations by walnut and carrot forms on carrot and weak infection on lupine.

Repeated inoculation tests with the California and New York isolates of the rose fungus showed that rootstocks of *Rosa odorata* and Manetti were very susceptible, *R. multiflora* moderately so, and Ragged Robin immune (Fig. 2). This is in line with the observation that the fungus has occurred in rose fields in central California (and presumably in other western states since it occurs on the stocks following digging) where the susceptible

² Culture supplied by Dr. L. M. Massey (10).

³ Culture supplied by Dr. D. E. Bliss from transfer of Peyronel's isolate (13).

⁴ Culture supplied by Dr. D. E. Bliss from Central Bureau voor Schimmelcultures transfer of Hamond's isolate (5).

⁵ Cultures supplied by Dr. R. W. Davidson (4, 6).

⁶ Culture supplied by Dr. D. E. Bliss (2).

⁷ Culture supplied by Dr. J. M. Walter (17).

⁸ Tests with stems from different plants gave inconsistent results indicating variability in resistance.

Manetti, *R. odorata*, and *R. multiflora* rootstocks are used, but has never been observed in southern California where the immune Ragged Robin is used. Use of an immune type of rootstock should prevent the increase of soil inoculum.

A study was made of the immunity of Ragged Robin rootstock. Treatment of hardwood stems in water at 100° C. for 10 minutes killed them and



FIG. 2. Relative resistance of 4 common rose rootstocks to *Chalaropsis thielavioides*. Bark was slit as in budding, stems dipped in spore suspension, and held in cutting frame for 20 days before being photographed. Left to right: Ragged Robin, *Rosa multiflora*, *R. chinensis* var. *Manetti*, and *R. odorata* in increasing order of susceptibility.

destroyed their resistance to the fungus. Similar treatment at 50° C. reduced callusing and sprouting, and enabled the fungus to grow moderately well. Exposure to moisture-saturated air at 50° C. for 10 minutes did not injure the stems and enabled the fungus to develop slightly; loss of immunity did not, then, result from leaching of an inhibitory substance. The susceptibility of stems of Manetti and *Rosa odorata* also was increased by such heating.

The fungus developed quickly in moist chambers on cut surfaces of scions of *Rosa odorata* in contact with Ragged Robin stems, and apparently developed slightly on the latter at points of contact. Resistance is not restricted to phloem and cortical tissues, for a completely peeled Ragged Robin stem is still immune. Wounded stems of *R. odorata* sprayed with a spore suspension and covered with macerated tissue or expressed sap of Ragged Robin stems were not protected from decay.

The addition of sucrose, calcium nitrate, or both to the spore suspensions sprayed onto wounded Ragged Robin stems did not promote infection.

These limited tests indicate that the immunity of Ragged Robin to the rose fungus is restricted to living cells, is decreased by a degree of heat not markedly harmful to the host, and is not due to a strongly diffusible substance.

TEMPERATURE RELATIONS

Studies (5) with the walnut, peach, and carrot forms on carrot agar showed no growth at 0° C., very little at 5°, slight at 12°, optimum at 18–30° (walnut 18–25°, peach 25–30°, and carrot 18°), very little at 33°, and none at 35° C. Longrée (8) found for the rose and elm forms on potato-dextrose agar that the minimum temperature for growth was 0–3.5° C., optimum 18–27.5°, maximum 30.5–33°, and that at 33° C. the fungus was killed in 35 days. The elm fungus caused (20) less root rot of Chinese elm at 1.7–3.3° C. than at 4.4–10.0° or 12.8–23.9° C.

To determine the relation of temperature to growth of the rose fungus on rose, stems of *Rosa odorata* were inoculated and held in moist chambers in the laboratory (about 25° C.) and in refrigerated rooms at 12.8°, 5.0° and 0° C. After 26 days stems at 25° C. were badly decayed; at 12.8° C. stems were blackened for 8 mm. and had abundant endoconidia and macrospores; at 5° C. the stems were covered with endoconidia (and after 79 days the stems were blackened); at 0° C. no fungus development could be seen (after 79 days there was a very sparse endoconidial development and no blackening).

It is apparent that if rose stems as cuttings or stocks are to be stored under conditions inhibitory to the fungus, a temperature as close to 0° C. (21) as practicable without injury to the host should be used. It has been suggested that Chinese elm plants should be stored at 1.7–3.3° C. to control root rot (20).

DISSEMINATION OF THE FUNGUS

The spread of the fungus with infected plant parts has been shown for rose (3, 8), elm (20), and walnut (5). That the endoconidia are adapted to dissemination in water is shown by their gelatinous consistency in mass and by their ease of wetting. The severity of the disease under the moist conditions of the grafting case is circumstantial evidence of water dissemination. Field stocks in California are grown from cuttings which root in place. The soil is pulled away from the crown prior to budding so that the bud may be

inserted close to the roots and thus give a short "neck." The resulting ditch is used for irrigation following budding and the water thus bathes the T cuts and bud shields of an entire row; this supplies an excellent mechanism for rapid spread of the fungus, particularly when irrigations are frequent.

There is strong circumstantial evidence that the fungus may be spread by the worker who does the budding. Because of the abundance and stickiness of the endoconidia it would be expected that they would be spread on the budding knife and the worker's hands or clothing. All of the fields involved in the occurrence of bud failure in Santa Clara County in 1940 had been budded by one worker and the evidence pointed to spread from field to field. Even though the introduced inoculum were scant, the rapid development of endoconidia and the frequent post-budding irrigations would quickly spread the fungus through the field.

The 1940 epidemic in field-budded roses perhaps is best explained by the fact that the cuttings of *Rosa odorata* for one field were buried in clay soil for several weeks after removal from the plants during the rainy season and before being planted in the field. This unusual treatment, necessitated by delayed field preparation, was particularly favorable to development of black mold. The grower reported black crusts on the end of many cuttings on removal from the pit, but apparently there had been no pronounced rooting failure of the cuttings, perhaps because of selection of uninjured stems. It was found (20) that storage of Chinese elm trees in silt loam was more conducive to *Chalaropsis* root rot than in sand, and that in heavy soil good drainage was required. Applying these facts to the control of the rose disease, the material used for packing rose rootstocks for eastern shipment should be porous and never excessively wet.

Because of the significance of field infection of wounds the soil carry-over of the fungus is important. Composite samples were gathered in September, 1941, from the top 2-3 inches of soil in one of the rose fields involved in the 1940 epidemic. This was made up in a heavy aqueous suspension into which peeled Manetti stems were dipped before incubation in a moist chamber. Results were negative for both the bait-sticks and checks. Other composite samples collected from the same field at 3-6-inch depth in October, 1941, were tested similarly and a small number (8 stems in 83) developed *Chalaropsis*; after standing for 6 days the suspension again was tested and 9 in 81 stems were infected. Bits of old stems of *Rosa odorata* from the field were held in moist chambers but no *Chalaropsis* developed; this was to be expected from the rapid replacement of the fungus by more successful saprophytes commonly observed in inoculated stems. There were viable spores present on the stems, however, as shown by rinsing them in water which was bait-tested as above, 1 stem in 101 developing *Chalaropsis*; after the wash water had stood for 7 days a second test gave 2 infected stems in 71. Checks dipped in tap water remained uninfected. Infested sand which had been used in a test in April, 1941, and subsequently held dry in the laboratory, was tested by the same bait method 195 days later. Of 69 stems, 29 devel-

oped *Chalaropsis*, indicating that many spores of the fungus were still alive. The checks remained uninfected. A similar test of the sand after 4 years showed the fungus to be dead. The bait technique should prove useful in determining whether a given field is infested by the fungus.

These tests indicated that the fungus will live over in field soil for nearly a year. It is, therefore, a surprising fact that Manetti cuttings taken in the winter of 1940-41 from the field of stock plants which had sustained such heavy budding losses and planted in a field only a few hundred feet away, had no evidence of the fungus in either stocks, or buds placed in them, during 1941. The disease has not been found since in field-grown roses in California.

CONTROL

Cultural Practices. Since rootstocks for greenhouse roses are grown largely on the west coast and some are grafted by commercial growers in other parts of the country, it is apparent that final control of the graft failure disease will involve cooperation of the growers. It has been stated (10) that "The problem is evidently one to be worked out where the stock is grown and packed. . . . Evidence to date indicates that the problem facing the nurseryman . . . should not be a difficult one." The use of western budded plants instead of locally grafted stock would effectively transfer this problem to the western grower and automatically control the disease. The increasing trend (7) toward budded plants may eventually eliminate losses in grafting frames and reduce the disease to infrequent budding failure in western nurseries. This is regarded as the most effective single control procedure for the disease.⁹ The budded type of plant yields as well as the grafted one (18), and can be planted over a longer period of the growing season (7).

A number of other practices would be helpful in reducing losses from this fungus. Cuttings should be planted in the field promptly after being taken from the stock plant. If it is necessary to hold them prior to planting they should be stored at about 0° C., and it would follow that rootstocks shipped east for grafting might be handled in the same way. Should it be necessary to store rootstocks or cuttings in sand or soil, the material should be as porous and well drained as possible; they should never be buried in a pit. It has been shown (21) that dormant rose plants kept best when stored at -1.1° C. in packing material of 36-40 per cent moisture content; no injury from freezing, breaking dormancy, or attack by molds was found under these conditions.

Since the fungus is a wound parasite, freezing and unnecessary mechanical injuries to the plants should be avoided.

Apparently the parasite can be spread by workmen, perhaps on tools, clothing, hands, etc., and careful sanitation is therefore advisable. In situations where the fungus is troublesome it would be a reasonable precaution

⁹ Dr. J. A. Milbrath has expressed views in correspondence in agreement with this, and Dr. D. B. Creager has indicated that many Illinois rose growers have discontinued grafting because of the disease.

to have workmen change to clean clothing, wash carefully, and disinfect all tools with 1 per cent formaldehyde (1-40). Similarly all cutting benches, grafting frames, or propagating houses should be sprayed with this solution prior to use. Hammond (5) reported that this procedure plus painting the stems with formaldehyde prior to cutting reduced graft blight of walnut from 95 per cent in 1930 to 7 per cent in 1933. It is necessary to sterilize sand or other material in the grafting frame following a diseased crop before it is again used. This should be done with steam or chloropicrin rather than formaldehyde because of the possibility that the latter may leave a persistent residue.

It would be a wise precaution not to replant roses in an infested field for several years, even though there was no recurrence of the disease in the single case reported here. Rotation of immune Ragged Robin with the susceptible rootstocks would prevent increase of inoculum of the fungus.

The use of rootstocks of Ragged Robin and possibly *Rosa lara* would aid in reducing losses from this disease. The former stock is commonly used for outdoor roses and might be worthy of consideration for greenhouses.

Propagation by high budding (16) into growing canes of rootstock later made into cuttings for field planting might reduce budding failure. Thus the bud shields would be placed in aerial stems which could hardly have been infested by the water-borne spores of *Chalaropsis*.

Chemical Treatment of Grafts or Buds. Various workers have attempted to inhibit development of *Chalaropsis thielavioides* on various host parts by the use of chemicals. Hamond (5) painted walnut stocks with formaldehyde 1-40 prior to grafting and was fairly successful in eradicating the fungus. Creager (3) used a 2-hour immersion of stock plants in various chemicals before potting; of these potassium permanganate (1 lb. to 25 gals.) and formaldehyde (1-320) were considered best. Wright (20) reported that in tests on chemical treatment of Chinese elm roots no satisfactory control was effected.

Attempts were made to find some fungicidal or fungistatic chemical non-injurious to the host cambium that might be used on rose grafts or buds to control the disease. Stems of Manetti, or less frequently *Rosa odorata* or *R. multiflora*, were used in the tests. Pieces of bark were removed from several (usually 6-12) stems which were then dipped in a suspension of endoconidia and immediately sprayed or dusted with the chemical or dipped in it. The stems were held in moist chambers or in cutting boxes of sand and observed for about a month for presence of the fungus. A few tests with grafted stems held in moist cases, and one limited field test, were made.

Of 74 different materials so tested, in over 110 different dosages and methods of application, only the following had fungicidal or fungistatic properties: Arasan (50 per cent tetramethylthiuramdisulfide—used as dust and in aqueous suspension at 1-200); Arasan plus 1-1000 Isothan Q15 (20 per cent lauryl isoquinolinium bromide); Arasan plus 2 per cent phenyl mercuric acetate in bentonite 1-1; Dithane D-14 (20 per cent disodium ethylene bisdi-

thiocarbamate) 1-100 plus zinc sulfate 1-400 and hydrated lime 1-800; Dow seed disinfectant No. 9 (zinc salt of 2, 4, 5 trichlorophenol); Dow seed disinfectant No. 10 (sodium salt of 2, 4, 5 trichlorophenol); Isothan Q15 1-100 and 1-400; malachite green 1-100; 4 per cent phenyl mercuric chloride in bentonite; sodium dimethyl dithiocarbamate 1-3.3. All of these materials, and many others neither fungicidal nor fungistatic, were injurious to host cambium and prevented its further growth. Attempts to inhibit the development of *Chalaropsis* by simultaneous inoculations of agar cultures and injured rose twigs with *C. thielavioides* and *Penicillium notatum* were unsuccessful.

It would appear to be very difficult to control the graft- or bud-failure diseases by chemical treatment at the point of union.¹⁰ Even though some chemical, inhibitive or lethal to the fungus and noninjurious to host cambium, eventually may be found, for the present control may best be obtained by cultural practices. The use of chemical dips for rootstocks would be advisable prior to grafting operations; formaldehyde 1-320 has been suggested (3). Because of the unimportance of the graft disease in California no studies were made of chemical dips for rootstocks.

SUMMARY

The black mold, *Chalaropsis thielavioides*, caused failure of bud unions in several rose fields, and of grafts in one greenhouse in California. It has not been found in southern California where roses are largely grown on immune Ragged Robin rootstocks.

Infected buds are quickly killed and blackened, and the cut surfaces of scion and stock are blackened and union prevented.

Of the common understocks, *Rosa odorata* and *R. chinensis* var. *Manetti* are very susceptible to the fungus, *R. multiflora* moderately so, and Ragged Robin immune. The immunity factor of Ragged Robin is restricted to living cells and can be decreased by moderate heat treatments. The rose form of the fungus is pathogenic to numerous other roses and woody plants. The lupine, walnut, and Chinese elm forms are nonpathogenic to rose.

The fungus is disseminated by infected plant parts, irrigation water, spattering water drops, soil, and by the worker. It survives in soil or buried plant parts for nearly a year. Infection occurs only through wounds and the fungus is aggressive only on dormant plant parts.

The graft-failure trouble could best be avoided by the use of budded roses, fungus-free rootstocks, sanitation of tools, frames, etc., and chemical treatment of stems prior to grafting. Bud failure is rare, and can be prevented by immediate planting of cuttings or, when necessary, by storing them at 0° C., by rotation with an immune crop, use of immune Ragged Robin rootstocks, by high budding into canes later made into cuttings, and by precaution against spread by workmen.

¹⁰ After this paper was in print a note by J. A. Milbrath appeared (Flor. Rev. 97(2509): 26-27. 1945) in which similar results with 28 fungicides were reported. It was suggested that infected cuttings be soaked 1 hr. in Ceresan (1 lb. per 100 gal. water) before planting, and that benches, tools, etc. be sprayed with copper sulphate (1 lb. per 5 gal. water) prior to use.

Preventive chemical treatments of cut surfaces of the host were unsuccessful because fungicidal and fungistatic materials injured host tissues.

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REACTION OF ONION VARIETIES TO YELLOW-DWARF VIRUS AND TO THREE SIMILAR VIRUSES ISOLATED FROM SHALLOT, GARLIC, AND NARCISSUS

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As a part of a study of the nature and relationships of viruses isolated from shallot (*Allium ascalonicum* L.), garlic (*A. sativum* L.), and narcissus (*Narcissus pseudonarcissus* L. var. King Alfred), 30 varieties or lots of onions (*A. cepa* L.) were inoculated with these viruses and with an isolate of onion-yellow-dwarf virus (5, 6, 7) (= *Allium virus* 1 K. M. Smith = *Marmor cepae* Holmes) obtained from potato onion (*A. cepa* var. *solanium* Alef.) from West Virginia in 1943.

The last named is considered to be the common or type strain of yellow-dwarf virus, namely, that originally described by Melhus *et al.* (6). Henderson (4), who quotes Giddings as stating that this disease was confined to potato onions when observed in West Virginia in 1916, considered a sample of the West Virginia virus a duplicate of the one under study in Iowa. Other isolates from onions by the writers, namely, one from New York from perennial tree onion (*A. cepa* var. *viniparum* Metz) supplied by H. A. Jones and two from Oregon, from commercial onions supplied by F. P. McWhorter, appear to be identical with the West Virginia virus. This admittedly small number of samples indicates that yellow-dwarf occurring in commercial onions is usually of this type, and that other types such as our isolates from shallot, garlic, and narcissus are at least not commonly encountered in onions in nature. An isolate from Everready onions received from England in 1944 by H. A. Jones also appears to be of the common yellow-dwarf type, agreeing with this in symptom expression in the test variety Ebenezer, and failing to infect Utah Sweet Spanish.

The shallot virus was isolated from commercial shallot supplied from Baton Rouge, Louisiana, by J. E. Welch in 1943. Of 13 other samples supplied by E. C. Tims from Louisiana, 10 were found infected with yellow-dwarf as shown by symptoms and by transfer to onion. In one sample of a strong-growing variety marked "Miller No. 1" only 2 of 16 plants were affected, and a virus-free line of this has been segregated. Symptoms in shallot are essentially as in onion, and readily recognizable, but Louisiana shallots are apparently acceptable commercially when affected although Green² (2) has shown that yield is markedly reduced by a disease very similar to this in England. In onions the symptoms of the shallot virus are similar to those of common yellow-dwarf, except for greater virulence

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as shown by more extreme dwarfing and folding downward of leaves. The shallot virus is probably a strain of yellow-dwarf, but comparisons of properties and other criteria are not yet complete. Only the one isolate from shallot has been compared in detail with yellow-dwarf, and it is not certain that the virulent strain is characteristic of shallot infections. This virus is readily transmitted from shallot to onion by mechanical inoculation and also by *Myzus persicae* Sulz. with the methods used for viruses that are nonpersistent in the vector.

The garlic virus was isolated from commercial late or Italian garlic submitted by F. P. McWhorter from Oregon in 1943. Two other samples of late garlic from Oregon and one sample of early or Mexican garlic and one of late garlic from California expressed 100 per cent mosaic symptoms when grown in our greenhouse. Indeed, all *Allium sativum* garlic seen by the writers has shown mosaic. Transfers from several garlic samples to onion have been accomplished by mechanical inoculation and by *Myzus persicae*, using the transfer technique adapted to viruses nonpersistent in the vector, but these transfers typically yield low percentages of infection. These isolates, including one from garlic collected in Lanchow, China, by T. P. Dykstra, appear to be similar in the test onion variety Ebenezer, but only one has been compared with yellow-dwarf in detail. Symptoms in onions are similar to yellow-dwarf symptoms in early stages, but there is much less folding down of the leaves in later stages and the disease is much milder in effect. F. P. McWhorter³ recalls collecting a mild disease of this order in onions in Oregon, but we have no further record of such a disease appearing naturally in onions. The status of the garlic virus is still uncertain, but it may prove to be a mild strain of onion-yellow-dwarf virus.

The narcissus isolate was made to Ebenezer onion from typical narcissus mosaic material supplied by F. A. Haasis. Transfer of this by *Myzus persicae* has succeeded only once in several attempts and the virus has not yet been returned to narcissus. Therefore this isolate is not necessarily narcissus-mosaic virus (3) nor necessarily a common invader of narcissus. No transfer from narcissus to onion by mechanical means has succeeded even under conditions that permit success with the garlic to onion transfer. Symptoms of the narcissus isolate in onions are similar to those of garlic mosaic, and the virus is evidently closely allied to the latter.

All virus cultures were maintained in Ebenezer onions, the four isolates being carried in parallel so that inoculum was of comparable age for each. In the writers' earlier tests, inoculation by juice and by *Myzus persicae* transfer produced similar results, varieties susceptible by one method proving susceptible by the other method also. Inasmuch as all four isolates are transferred with ease from onion to onion by the earborundum-leaf-rubbing method, this method was used in the variety tests here reported.

Onion varieties for testing were grown from seed supplied by H. A. Jones and planted September 21, 1944, except that two additional lots of Yellow

³ Personal communication.

Sweet Spanish and one of Utah Sweet Spanish were sown February 16, 1945, to confirm unexpected findings with the main plantings. Inoculations were begun in January and continued into May, or until infection with one or both of the more virulent viruses was complete. Some of the September-planted onions were inoculated six times, those planted in February three times.

TABLE 1.—*Reaction of onion varieties to yellow-dwarf virus and to three other isolates from shallot, garlic, and narcissus*

Variety	Previous reaction ^a to yellow-dwarf		No. plants infected ^b with virus strain from:			
	Belts ville	Iowa	Yel- low- dwarf	Shal- lot	Garlic	Nar- cissus
Australian Brown	S	S	10	10	7	6
Brigham Yellow Globe	S		10	10	10	10
California Early Red	S		7	10	7	6
Creole	S	S	5	10	3	2
Early Yellow Globe	S		10	10	8	9
Ebenezer	S	S	10	10	9	10
Golden Globe	S		10	10	10	9
Italian Red			8	10	10	9
Michigan Yellow Globe	S	S	10	10	8	9
Red Wettersfield	S	S	10	10	4	9
Southport Red Globe	S		10	10	6	8
Southport White Globe	S		10	10	9	6
Stockton Yellow Globe	S		7	10	4	4
White Portugal	S		10	10	8	8
Yellow Globe Danvers	S	S	10	10	6	6
Yellow Globe Danvers	S	S	10	10	10	9
Crystal Grano	I or S		10	10	8	4
Early Yellow Babosa			0	10	0	0
White Babosa			0	10	0	0
Utah Sweet Spanish	I	(River	0	9	0	0
White Sweet Spanish	I	side	2	10	2	0
Yellow Sweet Spanish (1943 seed) ^c	I	Sweet	6	10	4	7
Yellow Sweet Spanish (1943 seed) ^c	I	Spanish,	7	10	2	2
Yellow Sweet Spanish (1944 seed)	I	I)	0	9	0	0
Crystal Wax	I	S	0	10	0	0
Lord Howe Island	I		0	10	0	0
San Joaquin	I		0	10	0	0
Yellow Bermuda	I	(Bermuda	0	10	0	0
		Red, S)				
Amphidiploid var. Beltsville	I		0	0 ^d	0	0
Nebuka	I	I	0	0 ^d	0	0

^a S = Susceptible, I = Immune.

^b In each case 10 plants were inoculated.

^c Strain mixture in seed lot.

^d Subinoculations also negative.

The result of inoculating the four viruses into 27 varieties of onions and three duplicate lots is shown in table 1. It is evident that 10 of the 30 lots showed immunity to typical yellow-dwarf virus, and that all lots showing immunity to this also proved immune to the milder viruses from garlic and narcissus. In strong contrast, the shallot isolate infected all typical onion varieties exposed, and only Nebuka (*Allium fistulosum* L.) and an amphi-

diploid (*A. fistulosum* × *A. cepa*) proved immune to this virus. Nebuka is a nonbulbing species, used extensively in the Orient as a green onion. The amphidiploid tested here was from a hybrid of Nebuka by White Portugal onion, produced by H. A. Jones and associates, and about to be introduced as a green bunching onion under the varietal name Beltsville.

Henderson (4) reported only Riverside Sweet Spanish immune from yellow-dwarf among 36 onion varieties tested in Iowa, but also listed Nebuka as immune, not regarding this as an onion. Onion varieties have changed during the intervening 10 years, so that comparatively few of those listed by Henderson are included in our trials. Henderson lists Crystal White Wax and Bermuda Red as susceptible to yellow-dwarf. Crystal Wax and Yellow Bermuda as now carried by H. A. Jones have proved uniformly immune from this virus in our tests. It is not reasonable to assume that the Iowa outbreak was of the shallot type, for this strain readily infects all Sweet Spanish onions we have tested. Additional varieties susceptible to yellow-dwarf in Henderson's list and also in other trials at the Plant Industry Station, Beltsville, Maryland, are Bottle onion (= Scott County Globe), Mountain Danvers, Potato onion, and Southport Yellow Globe. The White Persian variety, not tested in Iowa, proved susceptible in our trials.

Several discrepancies appear in our own data for the Grano and Sweet Spanish varieties. In table 1 Crystal Grano appears fully susceptible to yellow-dwarf, and the related Babosa varieties immune. In previous trials two lots of Early Grano and one of Crystal Grano gave immune reactions, but one of Crystal Grano yielded 54 per cent susceptible, with the remainder insusceptible after five inoculations.

Sweet Spanish varieties have proved uniformly immune from yellow-dwarf in 18 previous trials, and Utah Sweet Spanish withstood repeated inoculation in a parallel experiment (1). In the present series (Table 1) one lot of Yellow Sweet Spanish and one of White Sweet Spanish yielded susceptibles, not only to the common yellow-dwarf virus but also to the milder viruses from garlic and narcissus. This Yellow Sweet Spanish from 1943 seed was replanted in February together with 1944 seed of this variety and seed of Utah Sweet Spanish for comparison. In this second trial of Yellow Sweet Spanish from 1943 seed, susceptibles again appeared, while the 1944 seed lot of this variety and the Utah variety yielded only immunes. The 1943 seed lot gave rise to nonglossy individuals as well as to semiglossy plants characteristic of Sweet Spanish types. It is therefore considered that this Yellow Sweet Spanish was a strain mixture not characteristic of the variety. A similar mixture may also be assumed to have been present in the White Sweet Spanish lot listed in table 1.

Inasmuch as commercial onion varieties are thus found to vary in reaction it is preferable to state that lines immune from yellow-dwarf have been found to occur in the Spanish-type varieties Burrell's Sweet Spanish, Colorado No. 6, Utah Sweet Spanish, White Sweet Spanish, and Yellow Sweet

Spanish, and also in Crystal Grano, Early Grano, Early Yellow Babosa, White Babosa, Crystal Wax, Lord Howe Island, San Joaquin, and Yellow Bermuda. It should be emphasized that all these lines that have been tested have proved susceptible to the shallot virus, only Nebuka and the Nebuka \times White Portugal amphidiploid variety Beltsville proving immune to the latter.

A study of the inheritance of immunity from yellow-dwarf, carried on through three seasons, has failed to provide a satisfactory genetic interpretation. It seems certain that immunity from typical yellow-dwarf could be incorporated into all onion varieties, but it is highly unlikely that the inheritance of immunity will prove simple enough to permit the breeder to proceed without repeated testing for yellow-dwarf reaction in successive progenies. Extensive series of subinoculations from inoculated plants in these studies indicate that symptom expression is reliable in all varieties provided that they are maintained in active vegetative growth.

It should be stated that shallots are grown commercially in the United States only in Louisiana. If these plants are brought into commercial onion districts for horticultural or genetic studies, it would be wise to start them from seed, in order to avoid the danger of extending the range of the shallot virus.

SUMMARY

Twenty-seven varieties of onions were mechanically inoculated with onion-yellow-dwarf virus and with related isolates from shallot, garlic, and narcissus. Ten variety samples proved immune from yellow-dwarf and also to the isolates from garlic and narcissus, while 17 varieties were susceptible to these three viruses.

All but Nebuka (*Allium fistulosum*) and an amphidiploid, Nebuka \times White Portugal, proved susceptible to the shallot virus. These two green-bunching types are immune to all four viruses under test.

All four viruses were transmissible by *Myzus persicae*.

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INFLUENCE OF NITROGEN NUTRITION ON SUSCEPTIBILITY OF ONIONS TO YELLOW-DWARF VIRUS

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In testing onion (*Allium cepa* L.) varieties and hybrids for reaction to onion-yellow-dwarf virus^{3, 4, 5} (= *Allium virus* 1 K. M. Smith = *Marmor cepae* Holmes) it was noted that low infection was associated with low nitrogen level in certain potting soils. In the season of 1944-45 a test of this apparent relation was made by growing 4 varieties of onions at high and low nitrogen levels in subirrigated benches filled with Haydite.

Varieties. Seed of 4 commercial varieties was furnished by H. A. Jones. Ebenezer was selected as a representative of the highly susceptible class, and Utah Sweet Spanish as an immune variety. Two varieties, Creole and Stockton Yellow Globe, were chosen as apparently resistant to but not immune from this virus, on the basis of previous testing in soil culture.

Source of virus. Onion-yellow-dwarf virus was isolated from naturally infected Multiplier onion (*Allium cepa* var. *solanum* Alef.) from West Virginia in March, 1943, and subcultured in Ebenezer. All inoculations were made by the carborundum-leaf-rubbing method, which has proved highly efficient for transfer of this virus under favorable test conditions.

Experimental design. Eight of nine cement bench sections, each 8 feet by 57 inches by 7 inches were filled with "B" grade Haydite, and the ninth with a good soil mixture, composed of 2 parts composted soil, 2 parts sand, 1 part manure, and 1 part muck. Seeds of 3 varieties were sown August 16, 1944, directly in these benches. Seeds of the fourth variety, Creole, were sown on the same date in thumb pots of the same two media, and shaded 2 hours daily with black sateen cloth until September 21 to avoid premature bulbing likely to occur in this variety under long-day illumination. The Creole plants were transplanted into the assigned bench sections on September 26. Four replications of high nitrogen level and 4 of low nitrogen level were randomized among the 8 Haydite-filled bench sections. In each of these 8 plots and the one soil plot 3 rows of each of the 4 onion varieties were grown, with plant counts per variety plot ranging from 37 to 64. The total number of onions in the experiment was 2,033, with a mean of 56.5 plants per variety plot.

All plants were watered from the top until August 30. At this date one-half the final nutrient level was supplied to each Haydite plot by pumping from the tanks twice daily.

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³ Melhus, I. E., C. S. Reddy, W. J. Henderson, and E. Vestal. A new virus disease epidemic on onions. *Phytopath.* 19: 73-77. 1929.

⁴ Smith, K. M. A text-book of plant virus diseases. 615 pp. London. 1937.

⁵ Holmes, F. O. Handbook of phytopathogenic viruses. 221 pp. Minneapolis. 1939.

Each tank had a volume of 198 liters of solution. On September 27, solutions were changed and full-strength nutrient levels were then maintained. Solutions were completely renewed at intervals of 4 weeks. At weekly intervals the nitrate content and ammonia nitrogen content were determined and restored to previous levels. Nitrate was determined by the phenoldisulphonic acid method using an Aminco Type F photometer. Ammonia was measured in the same instrument after nesslerization.

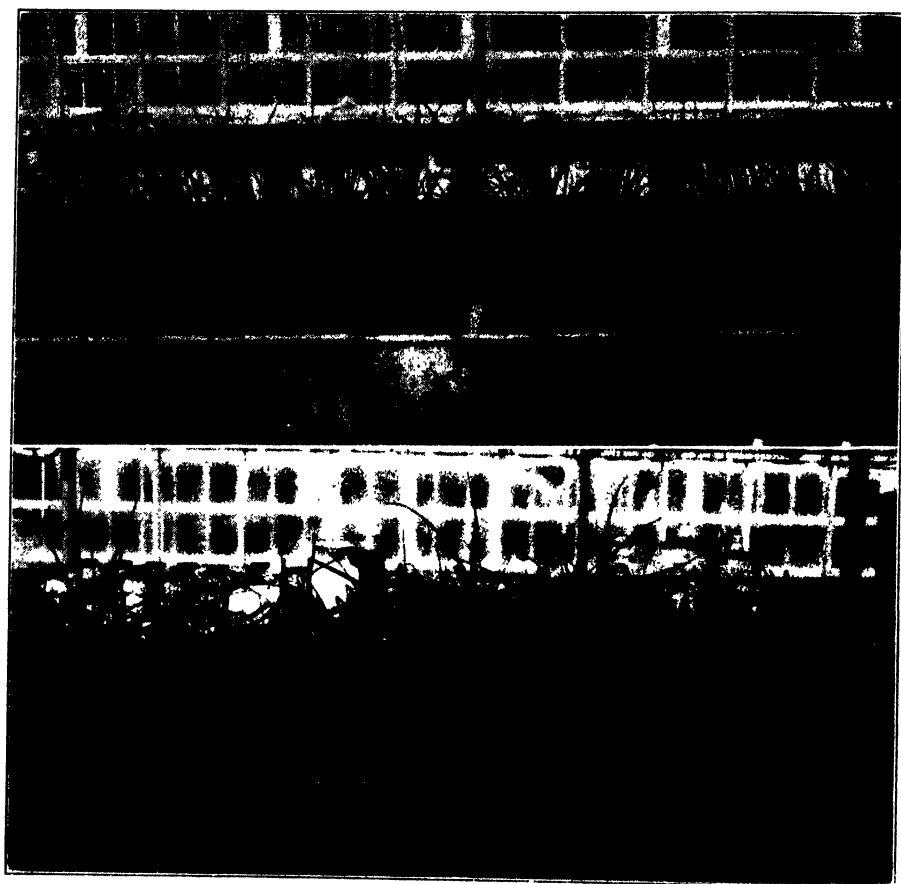


FIG. 1. Characteristics of growth of onions in Haydite culture December 6, 1944: A at low N level (5 ppm.); B at high N level (60 ppm.). Note that growth is normal at the low level, but much more vigorous at the higher level of nitrogen.

The low N nutrient solution contained the following millimolecular concentrations of six nutrient salts: $0.18 \text{ Ca}(\text{NO}_3)_2 \cdot 4 \text{ H}_2\text{O}$ [5 ppm. N and 7.2 ppm. Ca], $0.036 (\text{NH}_4)_2\text{SO}_4$ [1 ppm. N], $0.75 \text{ K}_2\text{SO}_4$ [58.6 ppm. K], $1.62 \text{ CaCl}_2 \cdot 2 \text{ H}_2\text{O}$ [64.8 ppm. Ca], $1.0 \text{ MgSO}_4 \cdot 7 \text{ H}_2\text{O}$ [24.3 ppm. Mg], and $1.0 \text{ KH}_2\text{PO}_4$ [31 ppm. P and 39.1 ppm. K]. The high N nutrient solution differed in that it contained 10 times as much $\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{ H}_2\text{O}$ and $(\text{NH}_4)_2\text{SO}_4$ but contained no $\text{CaCl}_2 \cdot 2 \text{ H}_2\text{O}$. Both low and high N solu-

tions also contained 2 ppm. of Fe, 1.5 of Mn, 0.5 of B, 0.15 of Zn, 0.06 of Cu, and 1 cc. per liter of approximately 0.1 N H_2SO_4 .

The high nitrogen level was chosen as favorable for vigorous vegetative growth of onions on the basis of previous studies of onion-seed production in Haydite culture. The low nitrogen level was chosen arbitrarily at one-tenth the high level, providing a solution capable of supporting less succulent but still fairly normal growth without extreme symptoms of nitrogen deficiency (Fig. 1). During the weekly intervals between analysis and restoration of solution levels, all the ammonia nitrogen was commonly absorbed or oxidized at both levels. The nitrate nitrogen sometimes dropped as low as 25 to 30 ppm. at the higher level and 2 to 3 ppm. at the lower level, but the proportional relation of 10 to 1 remained nearly constant.

Results. All plants were inoculated with a uniform preparation of yellow-dwarf-virus extract on October 24, and again on November 14. First symptoms were recognized in Ebenezer, Creole, and Stockton Yellow Globe

TABLE 1.—*Effect of high and low nitrogen levels in Haydite culture on susceptibility of onions to yellow dwarf, expressed as percentage infection. Means of 4 replicates compared with soil culture not replicated*

Variety	Haydite culture			Soil culture	
	High N, Dec. 5 (Col. 1)	Low N, Dec. 5 (Col. 2)	Means of columns 1 and 2	Low N to Dec. 5, High N to Jan. 3	Dec. 5
Ebenezer	99.7	86.3	93.0 ^a	87.6 ^a	96.9
Stockton Yellow Globe	41.6	20.3	30.8	27.7	40.0
Creole	40.2	17.9	29.0	25.5	30.0
Mean of 3 vars.	60.4 ^b	41.5	50.9	47.0	56.5
Utah Sweet Spanish	0.0	0.0	0.0		0.0

^a Differs significantly (odds 99 : 1) from means of other varieties.

^b Differs significantly (odds 99 : 1) from means of other treatments.

at the high N level and in soil on November 3, and at the low N level on November 7. On December 5 all recognizably diseased plants were removed from the beds and scored. The mean percentage infection for varieties and N levels is shown in table 1.

Variance analysis of the data for percentage infection (Table 1, Columns 1 and 2) on December 5 shows differences in yellow dwarf expression at the 1 per cent level between varieties and between N levels, but no significant interaction. Higher percentages of infection at the high N level than at the low level are significant within the resistant varieties Stockton Yellow Globe and Creole; differences are smaller and nonsignificant within the susceptible variety Ebenezer. The mean differences between the susceptible Ebenezer and the resistant varieties, Stockton Yellow Globe and Creole, are very highly significant, but there is no significant difference between the two resistant varieties. The plot of good soil closely approached the high N level plots in performance. The immune Utah Sweet Spanish was unaffected at either level of N. It is concluded that low N levels may significantly

hamper expression of yellow dwarf, that this effect is more likely to be encountered in varieties having a tendency merely to resist infection, but that nutrition levels show no tendency to break down immunity.

At this point it seemed of interest to determine whether the low infection observed at low N level represented an actually lower percentage infection or merely a failure to express symptoms. Accordingly, the low N level plots were raised to high N level on December 5, and so maintained without further inoculation until January 3. During this month at high N level 36 additional plants in those formerly low N level plots showed infection, a mean gain of 5.5 ± 2.03 per cent per variety plot, showing that additional plants previously infected were able to express symptoms at the high N level.

Variance analysis of the data for infection in low N plots January 3, one month after these were raised to high N level, in comparison with the December 5 data for high N level (Table 1, Column 1 vs. Column 4) shows the same general differences as the first analysis. Therefore a real difference is found between varieties and between N levels with respect to total infection, including infections that were not yet evident on December 5 at the low N level. Differences due to N level and to variety are thus highly significant with respect to percentage infection as well as to expression of symptoms.

The retarding or preventive effect of low N level is greatest, and highly significant, with respect to infection, but small and nonsignificant with respect to delay of expression (Table 1, Column 2 vs. Column 4). Admittedly, it was not determined whether further expression would have occurred in an additional month at low N level, or whether further expression would have occurred in the high N level plots without further inoculation, but such refinements could not have altered the conclusion that low N level depresses infection.

In commercial culture of onions, nitrogen will not often fall low enough to affect infection with yellow dwarf. However, in experimental pot culture in soil, nitrogen may become limiting and lead to low infection and expression. It is important, therefore, to maintain satisfactory N levels in such cultures when studying yellow dwarf effects.

RESISTANCE TO ONION YELLOW DWARF

Immunity to yellow dwarf in some Sweet Spanish varieties has long been known⁶ but the resistance of Creole and Stockton Yellow Globe has not been noted previously. Resistance is of minor interest to the breeder of new types when immunity is available, except that it may act as a confusing factor in interpreting inheritance of immunity. The present experiment provides evidence that these resistant onions differ sharply in response to yellow dwarf inoculation from the highly susceptible Ebenezer and also from the immune Utah Sweet Spanish.

⁶ Henderson, W. J. Yellow dwarf, a virus disease of onions, and its control. Iowa Agr. Exp. Stat. Res. Bul. 188. 1935.

It was considered desirable to determine whether a resistant variety is genetically uniform, so that all individuals are capable of infection, or whether evidence of segregation for immunity could be found. Accordingly all varieties in high N level plots were continued, and plants in the soil plot were transplanted to these on December 5 to increase the size of samples. These plots were maintained at high N level until June and inoculated 17 times in all, at intervals up to April 17, when bulbing and flowering tendencies made further inoculations unprofitable. The cumulative percentages of infection recorded at intervals are shown in table 2.

TABLE 2.—*Cumulative percentage of yellow dwarf infection in 4 varieties of onions grown at high N level in Haydite, resulting from 17 successive inoculations between October 24 and April 17*

Variety	Original number of plants	Cumulative percentage infection							
		Nov. 14	Dec. 5	Jan. 23	Feb. 27	Mar. 27	Apr. 17	May 15	June 11
Ebenezer	299	94.3	99.0	100.0					
Stockton Yellow Globe	261	30.3	41.4	70.5	80.1	88.1	92.3	92.7	93.1
Creole	296	18.2	37.8	58.4	68.6	79.7	83.8	83.8	83.8
Utah Sweet Spanish	289	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

The susceptible Ebenezer develops a high percentage infection promptly and the few escapes soon succumb to further inoculation. In the resistant Stockton Yellow Globe and Creole varieties 20 to 30 per cent are infected early, and the escapes gradually are infected in small rather uniform increments over several months and many exposures. It was not feasible in this study to demonstrate that all individuals of the Stockton Yellow Globe and Creole varieties are susceptible, but this seems more probable than that they show segregation for immunity.

It is also of interest to note that there was no breakdown of immunity in Utah Sweet Spanish at favorable high nitrogen level and under repeated exposure.

SUMMARY

Four varieties of onions grown at high (60 ppm.) and low (6 ppm.) weekly initial N levels in Haydite were inoculated with onion-yellow-dwarf virus. Both the percentage expression of symptoms and the percentage actual infection were lower at the lower N level, both differences being highly significant.

Immunity of the Utah Sweet Spanish variety was unaffected by repeated inoculation at a high N level that enhanced infection in both susceptible and resistant varieties. Two varieties, Creole and Stockton Yellow Globe, showed resistance to infection distinct from the immunity of Utah Sweet Spanish.

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THE RELATION OF SOIL TEMPERATURE TO THE DEVELOPMENT OF ARMILLARIA ROOT ROT¹

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Soil temperature is one of the important conditioning factors in the development of root diseases. Much of the literature on this subject has been summarized by Jones *et al.* (7), and, more recently, by Garrett (6). The relation of soil environment to the development of Armillaria root rot has, however, received little attention, though the causal agent, *Armillaria mellea* (Vahl) Quel., has been known for many years and the disease is of considerable economic importance. A series of experiments was undertaken to determine, if possible, the effect of soil temperatures on the growth of certain economic and ornamental species of plants, and on the development of Armillaria root rot on these plants.

The effects of soil temperature on plant growth are best studied under controlled conditions free from the diurnal and seasonal fluctuations of temperature characteristic of the natural soil environment. Modern thermostatically controlled equipment provides a practical means of obtaining nearly constant temperatures in soil for extended periods and over a comparatively wide range. The results of such experiments help to explain the geographic distribution of the disease and its seasonal development. Although the temperature of soil in nature cannot usually be controlled, exploratory studies of this kind serve as a guide in formulating effective control measures. Some of the cardinal findings of the first of the experiments have been given in a brief preliminary report (1). A complete, more detailed report is presented here.

MATERIALS AND METHODS

The effect of soil temperature on plant growth and disease development was studied in the greenhouse by means of five soil-temperature tanks (2, see fig. 22, p. 61), in which water baths were maintained at controlled temperatures. Seedlings or cuttings were grown in light sandy loam in metal containers, or pots, 8 inches in diameter and 12 inches tall, without holes for drainage. These metal pots were inserted through holes in the tank lids and submerged to a depth of 8.5 inches in the water baths. There were 8 metal pots in each soil-temperature tank.

Nine species of plants were used: Koethen and Homosassa sweet orange (*Citrus sinensis* [L.] Osbeck), Standard sour orange (*C. aurantium* L.),

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Sampson tangelo (hybrid of *C. paradisi* Macf. and *C. reticulata* Blanco), California pepper tree (*Schinus molle* L.), casuarina (*Casuarina stricta* Dry.), Lovell peach (*Prunus persica* Sieb. and Zucc.), Royal apricot (*P. armeniaca* L.), geranium (*Pelargonium hortorum* Bailey), and Ragged Robin rose (*Rosa* sp., var. Gloire des Rosomanes).

The citrus seedlings of experiments 1 and 6 (Table 1) were transplanted to small pots after one year in the seedbed, and were later transferred to the metal containers for the soil-temperature tanks. Stratified seeds of Lovell peach and of Royal apricot (experiment 2) were germinated in flats and transplanted to the metal pots. After one year's growth under uniform

TABLE 1.—*Temperatures of air and of water baths in greenhouse soil-temperature-tank experiments with Armillaria root rot*

Number, beginning date, and length of experiment	Air tem- perature in green- house, range	Water-bath temperature					Suscept	Plants in each tank	
		Tank I	Tank II	Tank III	Tank IV	Tank V		Non- inocu- lated	Inocu- lated
		° C.	° C.	° C.	° C.	° C.			
1. Nov. 3, 1938; 438 days	16.0-30.0	10	17	24	31	38	Koethen sweet orange	3	9
							Standard sour orange	3	9
2. Jan. 15, 1940; 218 days	21.0-27.0	10	17	24	31	38	Lovell peach	3	9
							Royal apricot	3	3
							Geranium	3	3
3. Mar. 31, 1941; 240 days	20.0-27.8	10	15	20	25	30	California pepper tree	6	14
4. Nov. 27, 1941; 181 days	21.0-24.4	8	13	18	23	28	Ragged Robin rose	2	6
5. Sept. 14, 1942; 324 days	16.7-26.7	7	12	17	22	27	Casuarina	4	6
6. Sept. 22, 1943; 385 days	16.1-23.9	7	12	17	22	27	Homosassa sweet orange	2	6
							Sampson tangelo	2	6

conditions, these plants were placed in cold storage for one month, before the beginning of the soil-temperature experiment, to induce dormancy. The geraniums (experiment 2) and Ragged Robin roses (experiment 4) were grown from cuttings. Seedlings of California pepper tree (experiment 3) and of casuarina (experiment 5) were first transplanted from flats to small pots and thence to the metal containers for the temperature tanks.

Each metal pot contained 1 to 3 plants, and weighed amounts of air-dried potting soil and water. When prepared, all containers in any one experiment had the same total weight and nearly uniform soil-moisture percentages. The soil was irrigated with distilled water to avoid excessive accumulation of salts. The soil-moisture content was maintained within the range between wilting percentage and field capacity by adding water at 2- to 7-day intervals to raise the total weight of each pot to a certain predetermined level. Small amounts of calcium nitrate or ammonium sulphate were applied

uniformly to all pots in any one experiment to maintain an adequate nitrogen supply. All plants were grown at first on a bench in the greenhouse under similar environmental conditions, before the beginning of the differential soil-temperature treatments. The plants were generally vigorous and reasonably uniform in size at the beginning of each experiment.

Temperature Control

The water in the five soil-temperature tanks was heated by electric soil-heating cable; that in three tanks was also cooled by means of electric refrigeration and circulated by means of pumps. After the plants had become established, the metal containers in which they were growing were inserted through the holes in the tank lids and partially submerged in the water baths. Except in experiment 1, a 2-inch layer of dry, granulated peat moss or cork was spread over the soil of each container for insulation immediately after inoculation. The soil at the surface, though reasonably well protected, was affected somewhat by the air temperature, especially if the temperature gradient was large. Differences up to 2° C. were noted between temperatures of the water baths and of soil in the surface-inch of submerged pots.

The temperatures of the air and of the water baths were recorded each day at 8 : 30 a.m., and at 1 : 00 and 5 : 00 p.m., to check the accuracy of temperature control. By means of thermostatically controlled steam coils and a desert cooler, the air temperatures of the greenhouse were maintained mostly between 21° and 27° C. The greenhouse was covered in summer with a coat of white paint to lower the air temperature and reduce radiation. The temperatures of the water baths ranged mostly within $\pm 0.5^\circ$ of the desired levels. Weekly mean temperatures of the air were calculated from thermograph charts by means of a polar planimeter (4, p. 56). Mean temperatures for each water bath were obtained by averaging 17 to 21 readings per week. With few exceptions, the performance of the control mechanism was satisfactory. The weekly mean temperatures of the water baths showed comparatively small ranges, the minus variation being never more than 0.9° and the plus variation being from 0.1° to 2.5°.

Inoculation

Stock culture B-508 of *Armillaria mellea* was used in the preparation of all inoculum for experiments 1 to 5, inclusive. This culture had been isolated October 22, 1937, from the sour-orange rootstock of a mature Valencia-orange tree near Azusa, California. Two strains of *A. mellea* were used in experiment 6: culture B-700, a reisolate of B-508, and culture B-701, a reisolate of B-677, which had been isolated July 3, 1941, from a peach root collected at Hamlet, North Carolina, and sent to me through the courtesy of Dr. J. S. Cooley.

Segments of citrus roots or pepper-tree stems, 1 to 2 inches in diameter and 7 inches long, were used in the preparation of the inoculum. The seg-

ments were sterilized in an autoclave, inoculated with *Armillaria*, and incubated at 20° C. until thoroughly invaded by mycelium and covered with the pseudosclerotium of the fungus. The segments were then inserted singly in the pots, in vertical holes made in the soil with a soil tube. Inoculation was done with the least possible disturbance of the soil or injury to the roots. Control plants were treated in the same manner but with sterilized root segments. The sterile segments used in the control pots in experiments 2, 3, and 4, had been previously inoculated with the pathogen. The inoculations, which were made a few days after the beginning of the differential temperature treatments, marked the beginning of the experiments (Table 1).

RESULTS

Effect of Soil Temperature on the Noninoculated Suspects

In experiment 1, the growth of Koethen-sweet-orange and Standard-sour-orange seedlings was greatly retarded at 10° C., and the new leaves were small and very chlorotic. Greatest top growth occurred at 31°, and the roots grew most rapidly at 17° to 31°. In experiment 6, all Homosassa-sweet-orange seedlings and Sampson-tangelo seedlings died at 7°; those at 12° were chlorotic and subnormal. Root development was similar to that of the tops, 12° being near the critical temperature for growth, and 17° to 27° being a favorable range. The mean dry weight of the tops was greatest at 27°.

All Lovell-peach seedlings, Royal-apricot seedlings, and geranium cuttings in experiment 2 died at a soil temperature of 38° C. Top growth of the peaches was greatest at 31° and least at 10°; root growth was greatest at 10° and 17°, however, and least at 31°. The apricots also developed the largest tops at 31° and the smallest at 10°, but root growth was greatest at 17° and least at 10°. The largest geraniums, including both tops and roots, developed at 17°, though the plants at 10° were nearly as large; the smallest living plants developed at 31°.

Experiment 3, with seedlings of California pepper tree, involved two different levels of soil moisture. Definite amounts of water and air-dried soil were mixed together when the seedlings were potted, so that soil in the so-called "dry" series contained 6.5 per cent moisture, and that in the "wet" series, 17.7 per cent, on a dry-weight basis. A uniform distribution of moisture was not maintained in the "dry" series, however, because the small amounts of water applied to the surface at frequent intervals were retained in the upper two-thirds of the soil mass, and moisture in the bottom was probably reduced to the wilting range. This situation adversely affected the growth of the tops and the weight of the roots. A soil temperature of 10° C. retarded top growth, but the increments in terminal growth and in diameter of stem were greatest, and of similar magnitude, from 15° to 30°. The root systems were relatively small at 10° and at 30°; they were larger at intermediate levels, and were at their optimum at 15°.

In experiment 4, with rooted cuttings of Ragged Robin roses, there was

a definite trend in vegetative growth of the tops, from weakest growth, at 8° C., to strongest, at 28°. The largest number of feeder roots occurred at 23°. The terminal growth rate of casuarina seedlings (experiment 5) was greatly retarded at 7° and most rapid at 27°. Root development seemed best at 12° and 17°; at 27° it was comparatively slight.

Effect of Temperature on the Pathogen

The relation of temperature to the development of rhizomorphs of *Armillaria mellea*³ was studied in the laboratory by growing the pathogen on 2 per cent potato-dextrose agar in large test tubes (Fig. 1). Twenty-one tubes



FIG. 1. Effect of temperature on the development of rhizomorphs of *Armillaria mellea* in 2 per cent potato dextrose agar. Incubated 11 days at the following temperatures: A, 10°; B, 14.6°; C, 19.7°; D, 24°; E, 27.4°; F, 31°; G, 36° C.

containing young, well-established fungus colonies were divided into seven groups of 3 each and incubated 11 days at 10°, 14.6°, 19.7°, 24°, 27.4°, 31°, and 36° C., respectively. Judging from the relative size of the rhizomorphs and the rate at which they penetrated the media, the most favorable range of temperature was 19.7° to 24°. Rhizomorphs of normal appearance, but retarded in growth rate, developed also at 10°, 14.6°, and 27.4°. Very little growth occurred at 31°, and none at 36°. Reitsma (8), in Holland, found that *A. mellea* in peptone nutrient solution produced greater dry weight at 25° C. than at 15° to 19°, or at 10°.

In the inoculation experiments in the greenhouse, all containers except those of the controls were supplied with segments of freshly prepared roots

³ Culture PB-41, isolated January 10, 1935, from citrus, Orange County, California.

TABLE 2.—Effect of soil temperature on *Armillaria* root rot of citrus seedlings, experiment 1

Soil temperature	Koethen sweet orange			Standard sour orange		
	Total plants tested	Plants attacked		Total plants tested	Plants attacked	
		Severely	Slightly		Severely	Slightly
°C.	No.	No.	No.	No.	No.	No.
10	9	4	0	9	6	1
17	9	1	2	9	3	4
24	9	0	2	9	0	0
31	9	0	0	9	0	0
38	9	0	0	9	0	0

or stems containing viable mycelium of *Armillaria mellea*. This inoculum contained abundant food reserves—probably more than enough to maintain viability throughout the longest experiment (438 days). Some pieces of inoculum in the “dry” series of experiment 3 may have lost viability because of undue drying, but for the most part, viability was lost only at soil temperatures of 30° C. and above. *Armillaria* in all the experiments was viable in inoculum after 181 to 438 days at temperatures of 7° to 28°, inclusive. The largest development of rhizomorphs, both in number and in length,

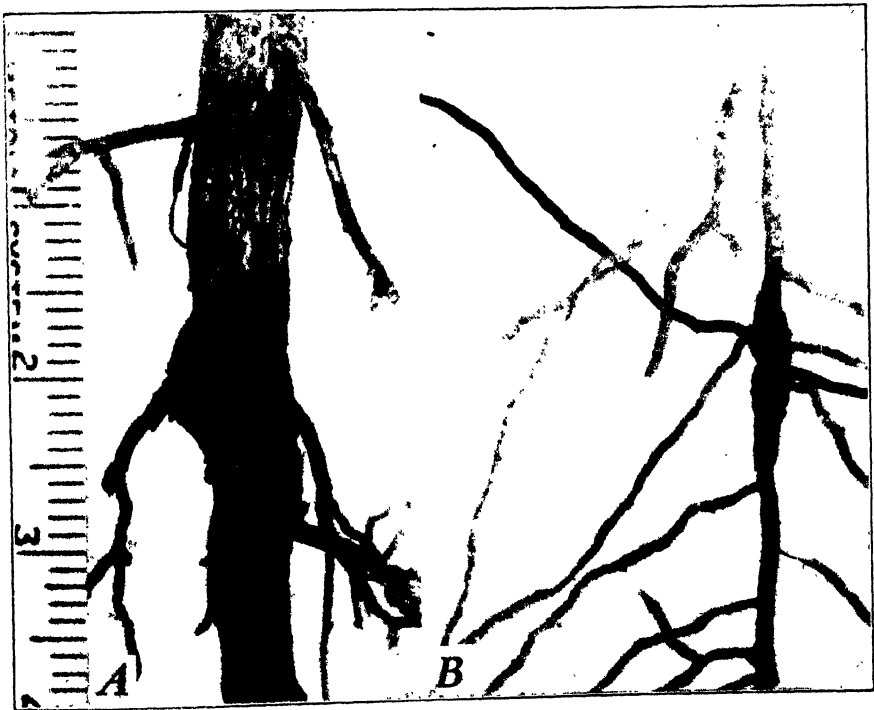


FIG. 2. Roots of Koethen sweet orange, experiment 1, showing lesions of *Armillaria* root rot which developed at 10°: A, taproot; B, branched feeder root. Note attachment of dark fungus rhizomorphs to the lesions.

occurred at the lowest temperatures, and the least development, at the highest. This surprising tendency was observed in all the experiments.

Effect of Soil Temperature on Pathogenesis

None of the noninoculated plants in these experiments was infected with *Armillaria* root rot. Among the inoculated plants, infection was limited to those at soil temperatures in which the pathogen produced rhizomorphs. As demonstrated by Thomas (9), infection was initiated with the penetration by a branch of the parent rhizomorph directly through the sound, healthy periderm of the susceptible. The method was similar in the different susceptibles, regardless of their relative susceptibility. Citrus seedlings were considera-

TABLE 3.—*Effect of soil temperature on Armillaria root rot of Homosassa-sweet-orange and Sampson-tangelo seedlings, experiment 6*

Soil tempera- ture	Culture B-700			Culture B-701		
	Total plants tested	Plants attacked		Total plants tested	Plants attacked	
		Severely	Slightly		Severely	Slightly
Homosassa sweet orange						
° C.	No.	No.	No.	No.	No.	No.
7	2	0	0	4	0	1
12	2	0	2	4	0	2
17	2	0	2	2	0	2
22	2	0	0	4	0	0
27	2	0	0	4	0	0
Sampson tangelo						
° C.	No.	No.	No.	No.	No.	No.
7	4	0	1	2	2	0
12	4	0	4	2	0	0
17	4	0	4	2	0	2
22	5	0	3	2	0	1
27	4	0	0	2	0	0

bly more resistant than the other plants that were tested. The citrus experiments, though comparatively long, were terminated before any plants had died or had shown marked secondary symptoms of *Armillaria* root rot. The effect of temperature on pathogenesis was therefore judged in citrus by the size and number of lesions produced.

In Koethen-sweet-orange seedlings, after 438 days, infection was comparatively severe at 10°, moderate at 17°, and slight at 24° C. (Table 2). Necrotic lesions on both the taproots and the small feeder roots were marked by the attachment of dark fungus rhizomorphs (Fig. 2). The lesions were swollen and contained considerable amounts of gum and also the typical white fungus fans of *Armillaria* in the bark and cambium. The presence of mycelium in the roots was also demonstrated by tissue cultures. Seedlings of Standard sour orange, under similar conditions, reacted much like those of sweet orange: the root rot was comparatively severe at 10° and moderate at 17° (Table 2).

In the citrus seedlings of experiment 6, the disease developed at temperatures of 7° to 22° C., but most rapidly at 12° and 17°. Homosassa-sweet-orange seedlings were not attacked at 22°, and were attacked only slightly at 7° (Table 3); but seedlings of Sampson tangelo were infected at all temperature levels, from 7° to 22°, inclusive, and appeared somewhat more susceptible than the sweet orange. The North Carolina strain of *Armillaria* (culture B-701) seemed to be nearly as pathogenic as the California strain (culture B-700) in this experiment, though in earlier, preliminary experiments with California pepper tree, poinsettia (*Poinsettia pulcherrima* Graham), and Royal apricot, it had seemed less pathogenic.

The relation of soil temperature to the development of *Armillaria* root rot was clearly demonstrated in experiment 2 (Table 4). Peaches, apricots, and geraniums, three very susceptible species, were infected at 10° C., but the disease progressed more rapidly at 17° and 24°, all the plants being

TABLE 4.—Effect of soil temperature on *Armillaria* root rot of Lovell peach, Royal apricot, and geranium, experiment 2

Soil temperature	Lovell peach ^a			Royal apricot			Geranium		
	Total plants tested	Plants attacked		Total plants tested	Plants attacked		Total plants tested	Plants attacked	
		Severely	Slightly		Severely	Slightly		Severely	Slightly
C.	No.	No.	No.	No.	No.	No.	No.	No.	No.
10	9	5	4	3	1	1	3	2	1
17	9	9	0	3	3	0	3	3	0
24	9	9	0	3	3	0	3	3	0
31	9	0	0	3	0	0	3	0	0
38	9	0	0	3	0	0	3	0	0

^a Delayed foliation affected 1 plant at 10°; 5 plants at 17°, and 3 plants at 24° C.

severely infected at these temperatures, and most of them dying during the experiment (Figs. 3 and 4). The plants at 31° and 38° escaped infection because the development of rhizomorphs was inhibited. At 38°, the plants died from excessive heat.

Large necrotic lesions developed in roots of both the peaches and the apricots, these lesions containing the characteristic mycelial fans of the pathogen and exuding copious amounts of gum (Fig. 5). Death occurred only when all roots were infected and the lesions had girdled the stem. The tops of the geraniums were not killed so easily: these plants, because of their succulent stems, remained alive even after the roots and the basal portion of the stem had died from effects of the disease (Fig. 6). A yellowish mottling, wilting, and necrosis of the leaf blades was noted (Fig. 6, B) during infection, and the older leaves dropped from the stems, leaving only a few of the youngest leaves at the apex. Even when the stem had been invaded and killed for several inches above the soil, the apical portion was alive and capable of generating a new root system.

Seedlings of California pepper tree, in experiment 3, had the following

numbers of infected plants (of the 7 inoculated plants in each lot) at the various soil temperatures:

Series	10°	15°	20°	25°	30° C.
"Dry"	2	4	4	4	0
"Wet"	5	7	6	6	0

Soil temperatures of 15° to 25° C., inclusive, were most favorable to the disease. Infection also occurred at 10°, but pathogenesis was slower. The

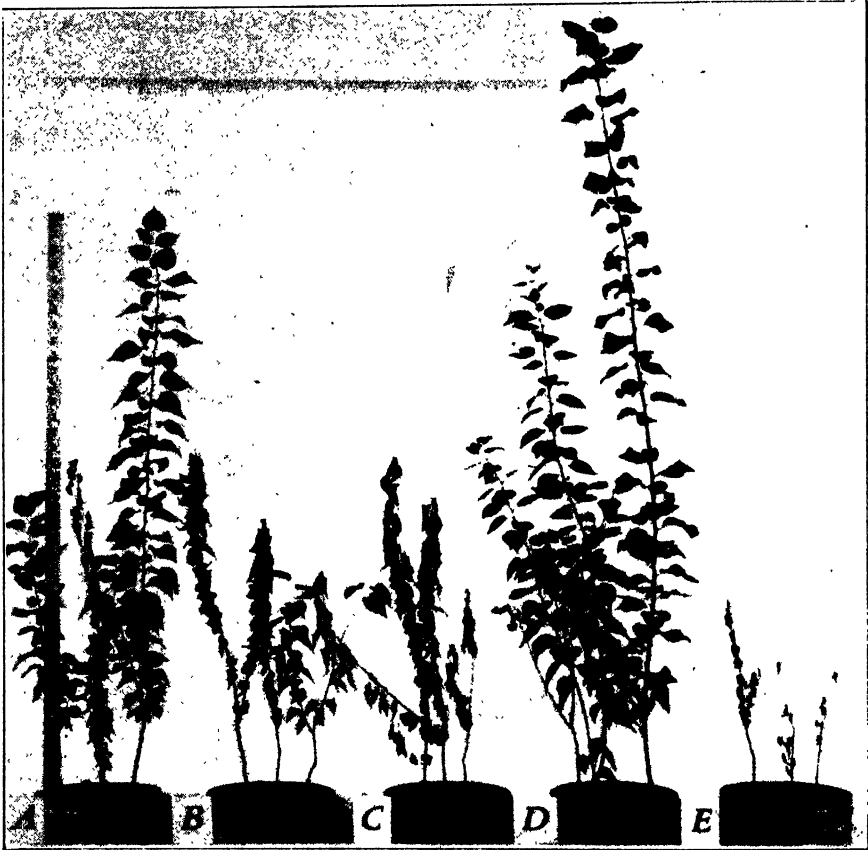


FIG. 3. Inoculated seedlings of Royal apricot, experiment 2, showing the effect of Armillaria root rot at different soil temperatures. A. Plants infected at 10° C., but injury moderate. B and C. Plants infected and dead at 17° and 24°, respectively. D. Large noninfected plants at 31°. E. Plants not infected, but killed by excessive heat at 38°.

plants at 30° were entirely healthy because growth of the pathogen had been inhibited. Secondary symptoms appeared first at 20° in diseased plants of the "wet" series, 67 days after inoculation, and at 25° in those of the "dry" series, 136 days after inoculation. Most root-rot lesions in the "dry" series were near the soil surface because dryness of the soil near the bottom of the containers had inhibited the growth of both roots and rhizomorphs. Soil

moisture, under these conditions, affected the rate of pathogenesis and also the percentage of infected plants. The symptoms observed during infection were rapid wilting and death following the complete girdling of the stem above the highest lateral root (Fig. 7).

In experiment 4, with Ragged Robin roses, the periods from inoculation until development of the first visible lesions, at the various temperatures, were as follows: 68 days at 18°, 77 days at 23°, 124 days at 13°, and 160 days at 8° C. Greatest injury from *Armillaria* root rot had occurred at 18°, when, after 181 days, the experiment was terminated: at this temperature 4 of the 6 inoculated plants were dead. Injury at 13° and at 23° was moderately severe, and at 8° it was moderate. All plants at 28° were strong and healthy. Of the 24 inoculated plants in the range 8° to 23°, inclusive, only

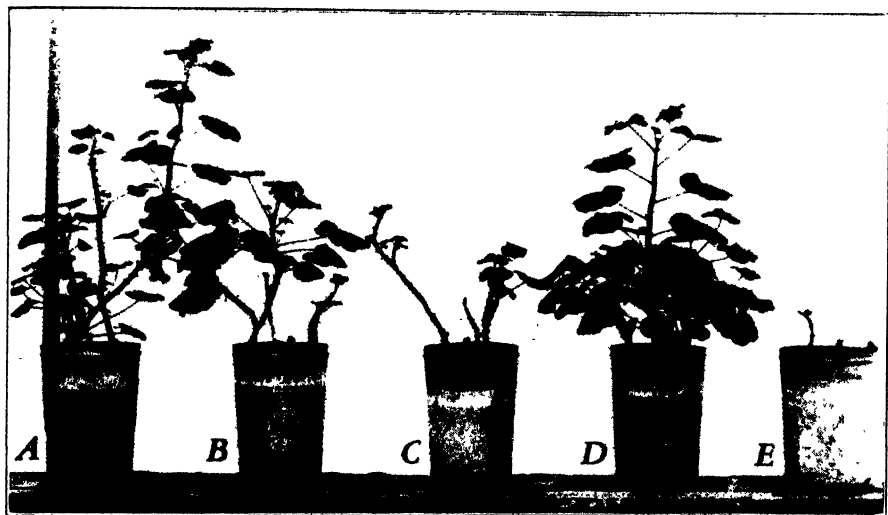


FIG. 4. Geraniums, experiment 2, showing the effect of different soil temperatures on the development of *Armillaria* root rot. A. Plants infected at 10° C., but injury moderate. B and C. Plants severely infected at 17° and 24°, respectively. D. Plants not infected at 31°. E. Plants not infected, but killed by excessive heat at 38°.

2 plants (at 23°) had escaped infection. Attack by rhizomorphs, which were most numerous at the lower temperatures, had produced many swollen and necrotic lesions on the roots and canes.

Casuarina seedlings, in experiment 5, showed the first visible symptoms of *Armillaria* root rot after 94 days at 22° C., after 164 days at 17°, and after 207 days at 12°. At the close of the experiment (after 324 days), there were dead plants at 12°, 17°, and 22°, but injury was most severe at 22°. Only slight to moderate injury was found at 7°. Of the 6 inoculated plants at each temperature level, all were infected at 7° and 12°, 5 were infected at 17°, 4 at 22°, and none at 27°. Typical primary symptoms were found, consisting of dark, necrotic, localized, swollen lesions in the stems and roots. The pseudosclerotium of the pathogen was visible as dark lines in longitudinal cracks in the bark. Some of the infected plants had survived in appar-

ently good condition by developing clusters of small lateral roots along the stem above the lesions.⁴

DISCUSSION

In the experiments reported in the present paper, pathogenesis occurred at soil temperatures of 7° to 25° C., inclusive. The maximum was between 25° and 27°; the minimum, probably slightly below 7°. The optimum, although broadly defined in most cases, varied from 10° to 25°. The reason



FIG. 5. Necrotic lesions of *Armillaria* root rot in roots of seedling plants of experiment 2: A, Lovell peach, at soil temperature of 24° C.; B, Royal apricot, at 17°.

for this variation was clarified somewhat when the temperature requirements of the different experimental plants were considered. The peach had the lowest optimum temperature range for root growth, and the rose the highest. When all nine species were arranged in this manner, they formed two groups (Fig. 8): A, those preferring the lower temperatures for root growth (10° to 17°); and B, those preferring the higher (17° to 31°). The first group included peach, casuarina, pepper tree, geranium, and apricot; the second,

⁴ In experiment 5 other plants of casuarina had been inoculated in a similar manner with *Clitocybe tabescens* (Scop.) Bres., from Florida. The results were entirely negative.

sweet orange, sour orange, Sampson tangelo, and rose. The optimum range for pathogenesis in group A was relatively high (15° to 25°), while that in group B was relatively low (10° to 18°). Thus, an inverse relation existed between the optimum ranges for root growth and for pathogenesis. In other words, greatest resistance to *Armillaria* root rot was shown by all these plants at temperatures most favorable to root growth.



FIG. 6. *Armillaria* root rot of geranium, experiment 2. A. Severely infected plant at soil temperature of 17° C. B. Mottling, wilting, and necrosis of leaf blades at 10° .

The healthy condition of inoculated roots at soil-temperature levels above 25° C. was apparently due to freedom from attack. At 26° to 31° , plants in group B were healthy and also had maximum root growth; plants in group A were healthy, but root growth was poor in that range of temperature. Roots of peach, apricot, geranium, and citrus were killed at 38° . In these experiments the maximum temperature for root growth in citrus was approximately 37° , and that for peach, apricot, and geranium was approximately 34° (Fig. 8).

Freedom from attack at these higher temperatures may be explained by the response of the pathogen. In agar the rhizomorphs of *Armillaria* grew slowly at 27.4° and very little at 31° C. In woody inoculum, buried in soil, the mycelium was viable after 181 days at 28°, but nonviable after 240 days at 30°. Since very few if any rhizomorphs were produced in soil at temperatures above 25°, plants grown in this range escaped infection because the means of disease transmission were lacking.



FIG. 7. Roots of California pepper-tree seedlings grown in soil at 15° C., experiment 3. A. Healthy control plant. B. Plant completely girdled and killed by *Armillaria* root rot. Some bark was removed to show the white mycelial fans of the pathogen.

The experiments on *Armillaria* indicate that the optimum temperatures for pathogenesis and for the growth of the pathogen are not always the same. A similar situation was discovered by Dickson (5) in regard to the seedling blights of wheat and corn, both of which are caused by *Gibberella saubinetii* (Mont.) Sacc. (optimum growth temperature, 24° to 28° C.). Wheat seedlings, growing better at low soil temperatures, were healthy at 8° but severely blighted at 16° to 28°. Corn seedlings, growing better at high soil temperatures, were severely blighted at 8° to 16°, but were healthy at 28°.

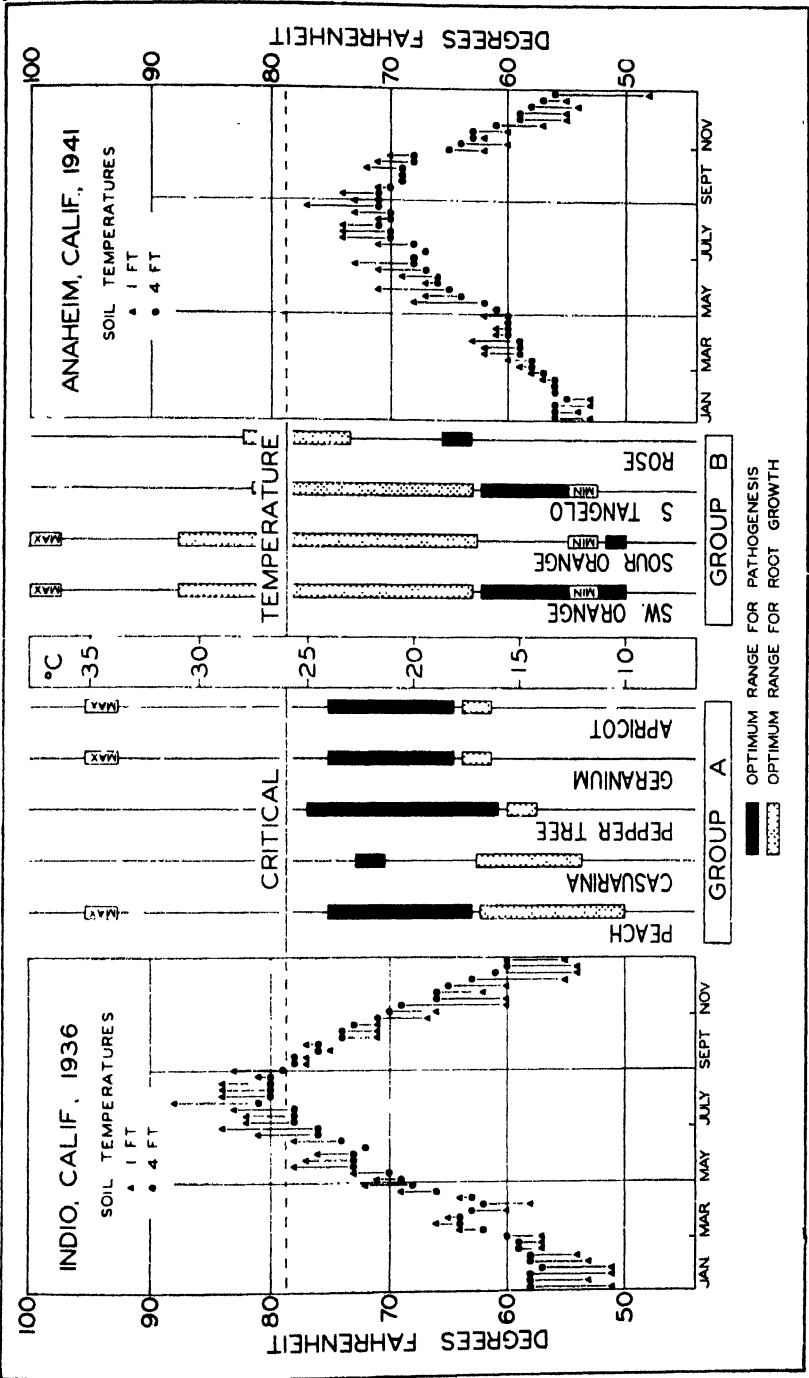


FIG. 8. Optimum temperature ranges for pathogenesis of *Armillaria* root rot and for root growth of nine species of plants used experimentally, compared with the march of soil temperatures (1- to 4-foot depths) at Indio, California, in 1936, and at Anaheim, California, in 1941. The minimum (min.) and maximum (max.) temperatures for root growth are indicated, where known. A critical temperature, above which pathogenesis did not occur, is tentatively indicated at 26° C. (78.8° F.).

The critical temperature for the seedling blight of wheat was 12°; that for seedling blight of corn was 20° to 24°. Dickson (5, p. 867) concluded that "the influence of environmental factors on the hosts seems to be the fundamental cause of susceptibility to the disease. The seedlings become susceptible when they are unable to respond favorably to the environment."

"Critical temperature," as used by Dickson (5), is taken to mean the boundary between two contiguous ranges of soil temperature, one in which pathogenesis occurs, and one in which it does not occur. Actually, in any suspect there are two critical temperatures for a disease, namely, the temperatures at the two extremes of the range for pathogenesis. For instance, the critical temperatures for *Armillaria* root rot in the California pepper tree are thought to be approximately 4° and 26° C., respectively.⁵ The first is of minor importance because it probably lies below the minimum temperature for root growth; the second is important because the pepper tree is known to thrive in soil up to 30°. The disease may have slightly different critical temperatures in each of the nine species used in the present experiments, but in the absence of more exact information, the upper critical temperature is tentatively placed at 26° in all cases (Fig. 8).

From these considerations, relative susceptibility of the experimental plants should be defined in terms of soil temperature. All the plants were presumably immune above 26° C. Plants of group A were comparatively more susceptible at 22° than at 10°. Considering only the rate of pathogenesis in its optimum range in each suspect, the relative susceptibility in these plants may be described as follows: geranium and apricot, very susceptible; peach, pepper tree, rose, and casuarina, susceptible; and Sampson tangelo, sweet orange, and sour orange, moderately resistant.

There is an element of chance in the time and place of contact of roots and rhizomorphs. Under favorable conditions, the probability that infection will occur in a given time and space depends in part on the product of the respective concentrations of roots and rhizomorphs.

The longevity of an infected plant depends not only on its size and relative susceptibility, but also on the location of the lesions. A plant will die quickly if the main stem is girdled by lesions, but if the lesions are at the extremities of the roots, no secondary symptoms of the disease may appear for many months.

In southern California, *Armillaria* root rot is prevalent throughout the orange- and lemon-growing districts, which extend 10 to 55 miles inland from the coast. The disease has not been reported, however, from the hot desert valleys east of the San Jacinto mountains, where dates and grapefruit are grown. Studies have been made on the temperature of irrigated soils at Indio (4), in the desert region, and at Anaheim (3), in the coastal region (See fig. 8). At Indio, in 1936, the mean weekly temperatures of soil, at 1- to 4-foot depths, reached a maximum range of 81° to 88° F. (27.2° to 31.1° C.), while at Anaheim, in 1941, the maximum range at these depths was only 71° to 77° F. (21.7° to 25° C.). Since these data are typical of

⁵ Rhizomorphs of *Armillaria mellea* grow very slowly in agar at 5° C.

their respective stations, soil in the principal root zone (1 to 4 feet) at Indio is largely above the upper critical temperature for *Armillaria* root rot about three months each year, while the soil of corresponding depth at Anaheim is usually not heated to this temperature. It seems probable, therefore, that excessive heat in the desert region is an important factor in preventing the development of this disease. The failure of certain artificial inoculation experiments at Riverside may also be attributed to high summer temperatures. Pathogenesis appears to be strongly influenced by seasonal fluctuations in soil temperature. From the data presented, pathogenesis in peach, casuarina, pepper tree, geranium, and apricot would be expected to develop most rapidly in the coastal region from spring to fall, while that in citrus and rose would develop most rapidly from late fall until spring.

SUMMARY

This paper reports a series of six soil-temperature experiments with *Armillaria* root rot on nine economic and ornamental species of plants, including Koethen and Homosassa sweet orange, Standard sour orange, Sampson tangelo, Lovell peach, Royal apricot, geranium, California pepper tree, Ragged Robin rose, and casuarina. The plants, potted in soil and well established, were inoculated with the pathogen, *Armillaria mellea* (Vahl) Quel., and grown 181 to 438 days, in the greenhouse, in five soil-temperature tanks. The water baths in these tanks were held at different, controlled temperatures ranging from approximately 7° to 38° C. Air temperatures in the greenhouse ranged mostly between 21° and 27°.

Rather wide differences were observed in the responses of noninoculated plants to soil temperature. Optimum temperatures for root growth in peach, casuarina, pepper tree, geranium, and apricot (group A) were 10° to 17° C., inclusive, while those for citrus and rose (group B) were 17° to 31°, inclusive.

In citrus, top growth was greatly retarded at soil temperatures of 10° to 12° C., the new leaves being small and very chlorotic; largest top growth occurred at 27° to 31°. The minimum temperature for root growth was 12°; the maximum, slightly below 38°.

All plants of peach, apricot, and geranium died at a soil temperature of 38° C. Top growth of peaches and apricots was greatest at a soil temperature of 31° and least at 10°; that of geraniums was greatest at 17° and least at 31°. Top growth of pepper trees was retarded at 10° but was vigorous and of nearly equal magnitude at 15° to 30°. The growth rate of roses increased from 8° to 28°. Top growth of casuarina was greatly retarded at 7° and most rapid at 27°.

The rhizomorphs of *Armillaria mellea* grew most rapidly in sterile, deep nutrient agar at 19.7° and 24° C. Rhizomorphs of normal appearance but retarded in growth rate developed at 10°, 14.6°, and 27.4°. Very little growth occurred at 31°, and none at 36°. Slight growth has also been found at 5°. The pathogen remained viable in inoculum at controlled tempera-

tures from 7° to 28°, inclusive, during 181 to 438 days. After these long periods, greatest development of rhizomorphs in nonsterile potting soil was evident at the lowest experimental temperatures. The apparent inconsistency of the effect of temperature on the development of rhizomorphs in agar and in soil is not explained.

Pathogenesis in test plants was observed at soil temperatures ranging from 7° to 25° C., inclusive. The maximum temperature was between 25° and 27°; the minimum, somewhat below 7°. The optimum range for pathogenesis in plants of group A was 15° to 25°, while that for plants of group B was 10° to 18°. Greatest resistance was shown by all plants at temperatures most favorable to root growth.

There are two critical temperatures for *Armillaria* root rot in any susceptible: one at each extremity of the range for pathogenesis. The lower temperature is of minor importance, usually lying below the minimum for root growth, but the upper one is important, because many susceptibles thrive at higher temperatures, at which they are free from attack. The disease may have slightly different critical temperatures in each of the nine species of plants here tested, but in the absence of more exact information, the upper critical temperature is tentatively placed at 26° C. in all cases.

In southern California the prevalence of *Armillaria* root rot throughout the coastal region, and its supposed absence in the inland desert areas, are apparently related to differences in soil temperature. Soil at Indio (desert region), at 1- to 4-foot depths, is largely above 26° C. for about three months every year; soil of corresponding depths at Anaheim (coastal region) seldom reaches this temperature. In the coastal region, pathogenesis in peach, casuarina, pepper tree, geranium, and apricot would be expected to develop most rapidly from spring to fall, while that in citrus and rose would develop most rapidly from late fall until spring.

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PHYTOPATHOLOGICAL NOTES

*Bud Blight of Soybean Caused by the Tobacco Ring-Spot Virus.*¹—A disease of soybean caused by the tobacco ring-spot virus has been responsible for substantial losses in yield in the midwestern producing areas in recent years. It is not definitely known how long significant damage has been occurring but the losses in 1943 and 1944 exceeded all previous records and ranks this disease among the most destructive of the soybean.

Pierce² noted the destructive nature of this virus on soybean and certain other legumes, but did not observe its occurrence in nature. Samson³ reported finding the disease in experimental plantings of vegetable soybeans in Indiana in 1941. Melhus⁴ observed it in Iowa in 1942, and later Johnson⁵ reported the disease on soybean in Ohio. It is likely that at that time, it was distributed extensively throughout the midwest in small amounts but had escaped detection.

In view of the increasing importance of this disease and the variety of symptoms which have come to be associated with it, it seemed desirable to report at this time the information which is available. This paper is concerned primarily with the identification and description. Control measures have not been found. Studies are underway at the U. S. Regional Soybean Laboratory to find means of combating this menacing disease.

Symptoms. The effect of the tobacco ring-spot virus on the soybean plant depends to a large extent upon the age of the plants at the time of infection. Like many virus diseases, the greatest effect on the host is in the young growing tissues.

Natural infection in the field is rarely observed before the plants are 7 to 8 weeks old. In Illinois, therefore, the disease appears in late July or early August. At this time, the symptoms consist of a characteristic curving of the terminal bud apparently due to unilateral elongation, as shown in figure 1. A. The young expanding leaves have a bronzed appearance and sometimes a few leaves drop from the plant. The pubescence on the young stem tip becomes more prominent than usual and may darken somewhat. Eventually the growing point becomes necrotic and assumes a brittleness which causes this portion to break off easily when touched. When the bud symptoms first appear, a careful search may disclose areas of reddish brown discoloration in the pith of the stems or branches, appearing first in the

¹ A publication by the U. S. Regional Soybean Laboratory, a cooperative organization participated in by the Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration; and the Agricultural Experiment Stations of Alabama, Arkansas, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Louisiana, Michigan, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, North Dakota, Ohio, Oklahoma, South Carolina, South Dakota, Tennessee, Texas, Virginia, and Wisconsin.

² Pierce, W. H. Viruses of the bean. *Phytopath.* **24**: 87-115. 1934.

³ Samson, R. W. Tobacco ring spot on edible soybeans in Indiana in 1941. *U. S. Dept. Agric., Plant Disease Reporter* **26**: 382. 1942.

⁴ Melhus, I. E. Soybean diseases in Iowa in 1942. *U. S. Dept. Agric., Plant Disease Reporter* **26**: 431-432. 1942.

⁵ Johnson, Folke. Soybean streak in Ohio. *U. S. Dept. Agric., Plant Disease Reporter* **27**: 86-87. 1943.

vicinity of the nodes. Plants becoming infected at this stage seldom produce seed. However, they remain green, fail to ripen normally, and persist in the field until frost. The pith discoloration gradually increases until late in the season when the pith in the entire stem and branches may be discolored. Necrotic areas may extend to the exterior of the stem in some cases, particularly at the nodes. Streaking of the petioles and large leaf veins occasionally has been observed.

Symptoms resulting from infection near blossoming time when most of the terminal elongation has ceased present a somewhat different picture. The expanding tissues in the flowers and young pods are the main points



FIG. 1. A. Soybean plant infected with bud blight showing the characteristic curving of the terminal bud. B. Pod symptoms resulting from infection near blossoming time. Note distorted and shrunken pods. (Photograph B was furnished by courtesy of Dr. B. Koehler of the Illinois Agricultural Experiment Station.)

affected. This type of infection causes the greatest losses commercially since it is usually very extensive in the affected fields and causes considerable reduction in the amount of seed produced. A high percentage of the young pod clusters may wither and fall off within 10 days after infection. The most common symptom, however, is the dark blotching effect on the pods as illustrated in figure 1, B. Many of the pods that do not fall are shriveled and produce defective seed or none at all.

Soybeans that do not ripen normally often produce seed of very poor quality. Samples of seed from healthy and bud-blight infected plants are shown in figure 2. The physiologic conditions bringing about this poor seed quality may be brought on by other factors, as well as infection by this virus.

This condition, therefore, should not be invariably associated with the bud-blight disease.

Identification of the Virus. Symptoms produced on tobacco by this virus are characteristic of tobacco ring spot. Tobacco plants which have "recovered" from the initial stages of the disease and are symptomless do not again show symptoms when reinoculated with the soybean strains or strains from tobacco definitely known to be tobacco ring-spot virus. The converse is also true, that is, tobacco plants which have "recovered" from symptoms induced by known tobacco ring-spot virus, produce no further

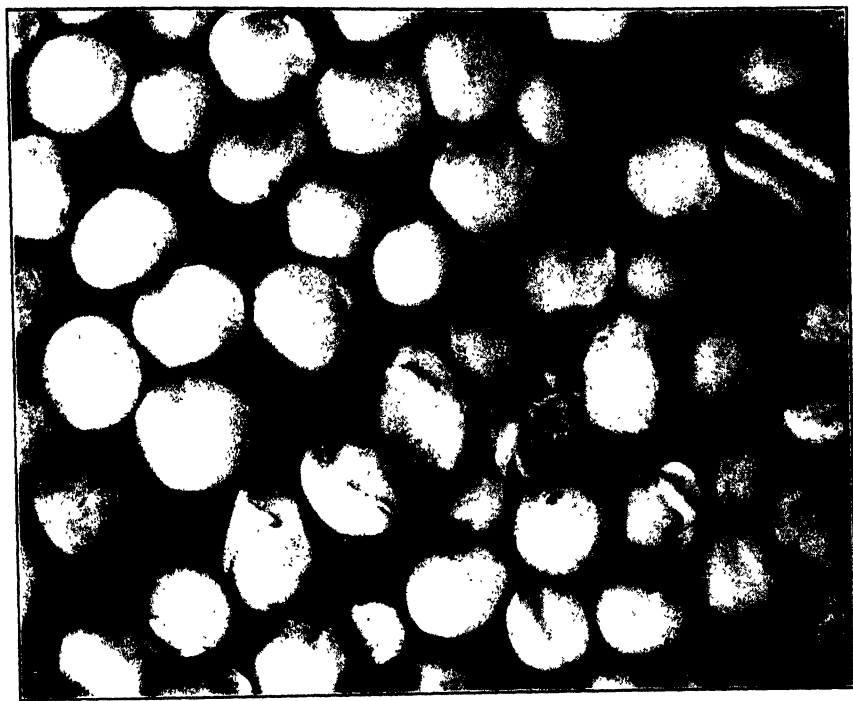


FIG. 2. Normal seed (left) of Bansei soybeans, and damaged seed (right) from artificially infected Bansei soybeans grown in adjacent rows in the field.

symptoms when inoculated with strains from soybean. Healthy plants of the same age become severely diseased upon inoculation. The soybean and tobacco strains were compared on garden bean (*Phaseolus vulgaris* L., var. Red Kidney). In both cases the plants were severely stunted and eventually succumbed to a progressive necrosis that usually started from the point of inoculation. Usually local necrotic lesions were produced upon the inoculated leaves. Symptoms on cucumber (*Cucumis sativus* L.) were indistinguishable with strains from soybean and tobacco. In both cases severe stunting and mottling continued indefinitely.

The thermal inactivation point corresponds exactly to that recognized for the tobacco ring-spot virus which is around 65° C. for 10 minutes. In

all the inactivation tests virus from soybean and known tobacco ring-spot virus were treated simultaneously.

Discussion. The occurrence of the tobacco ring-spot virus on soybean in the Midwest may help investigators to determine its mode of transmission. The agency accounting for its rapid spread through soybean fields is very likely one of the insects commonly associated with this crop. Typically the most damaging phase of the disease, *i.e.*, the pod spotting and blighting, occurs around the margins of the fields first and can be observed to progress inward as the season progresses. An intensive search is being made at the present time at this laboratory in an attempt to find an insect vector.

Summary. The symptoms produced on soybean by the tobacco ring-spot virus are described and illustrated. The identification of the virus by others was verified by thermal inactivation tests, "plant immunity" tests, and symptomology. The possibility of the occurrence of an insect vector is discussed.—WILLIAM B. ALLINGTON, Associate Pathologist, U. S. Regional Soybean Laboratory, Urbana, Illinois.

*Two Aids for the Study of Potato-Late-Blight Epidemiology.*¹—An accurate measure of viable inoculum and an evaluation of environmental conditions that influence infection and the development of disease are essential to the study of plant disease epidemics.

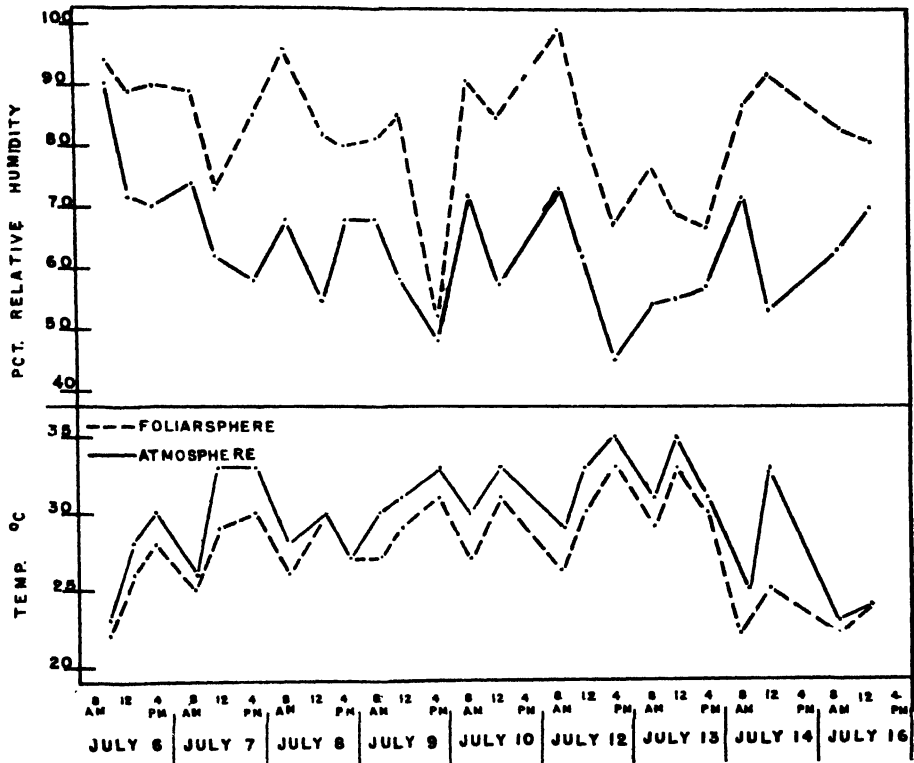
The presence in the air of sporangia of *Phytophthora infestans* can be determined fairly readily by exposing vaselined microscope slides which are subsequently examined with a microscope. Stem-rust inoculum has been measured successfully in this way, but the method has some disadvantages when used for late blight. First, the sporangia of late blight cannot readily be distinguished from those of several other downy mildews. Second, they are killed by short exposures to dry air, and, although the number of sporangia (in the absence of similar species) could be determined with a fair degree of accuracy with slides, assuming that slide exposures are an accurate criterion of the number falling on plants, the number capable of infection cannot be accurately measured.

In 1943 the writer exposed potted potato plants in several fields near St. Paul and Minneapolis, Minnesota. These plants had been raised in a greenhouse where fairly high temperatures as well as some protection from the outside air reduced the possibility of accidental infection. Check plants indicated that the possibility of accidental infection was zero. These plants were exposed in various locations, either overnight or for 4-hour periods during the day. At the end of the exposure period they were placed in portable moist chambers in an automobile and brought to the laboratory where they were incubated at about 70°F. and 100 per cent relative humidity. Control plants, directly from the greenhouse, were incubated at the same time and under the same conditions. None of the control plants became infected.

¹ Paper No. 2268, Journal Series, Minnesota Agricultural Experiment Station.

Late-blight lesions usually began to appear within 3 or 4 days after the plants were exposed. The lesions were counted, and their numbers, together with the numbers of sporangia found on slides exposed at the same time, were used to determine the "blight infection potential."

Having available an estimate of the viable inoculum at a given location, predictions regarding infection will depend upon an accurate knowledge of environmental conditions affecting germination and infection. Numerous attempts,^{2,3} have been made to correlate weather data with late blight epidemics, and some workers^{4,5} have found that mean temperature and precipi-



tation data are insufficient for the purpose. Total precipitation, if it comes as quick, heavy showers, may still be unfavorable for late blight if the intervening periods are dry. Prolonged periods of high humidity, however, may provide very favorable conditions for the disease even though the total precipitation be low.

It is suggested that ordinary weather observations may not be satisfac-

² Lutman, R. F. Twenty years' spraying for potato diseases. Potato diseases and weather. VI. Agr. Exp. Stat. Bull. 159. 1911.

³ Martin, W. H. Late blight of potatoes and the weather. N. J. Agr. Exp. Stat. Bull. 384. 1923.

⁴ Gratz, L. O. Disease and climate as pertaining to the Florida and Maine potato sections. Phytopath. 20: 267-288. 1930.

⁵ Orton, C. R. Meteorology and late blight of potatoes. (Abstr.) Phytopath. 6: 107. 1916.

tory for this purpose because such measurements are usually not made in the environment where infection occurs, *i.e.*, among the leaves of the potato plant. The observation that blight is often most severe in low spots in a field is evidence of the importance of temperature and humidity in limited environments rather than several feet from the ground and in open spaces where weather stations are usually located.

To compare the meteorological conditions in the atmosphere with those among the foliage of the plants (referred to as the "foliarosphere"), temperature and humidity were measured among the leaves of potato plants in the field and also at a point 5 feet above the plants. The relative humidity was measured by means of dew-point apparatus. The data (Fig. 1) show that although temperatures in the two locations differed but slightly, the differences in relative humidity were often very great. The differences were striking on July 8, 12, and 14.

There was 0.3 inch of rain on July 2 and again on July 12. Late blight was found in the field on July 7 and increased from then until July 19. Standard meteorological observations during this period indicate that the weather was unfavorable for blight development. On the other hand, the higher relative humidities in the foliarosphere indicate that among the leaves of the plant, moisture conditions favorable for fructification of the fungus and germination of inoculum probably obtained for sufficient periods for these processes to occur.

The data presented are preliminary in nature, and are not sufficient to show conditions at all hours of the day. Nevertheless they do illustrate the importance of the microclimate as a factor in the epidemiology of late blight and that potted potato plants exposed in fields when late blight inoculum is present may be used as indicators of the "blight infection potential."—W. D. THOMAS, JR., University Farm, Saint Paul, Minnesota.

*A New Bean Mosaic in Idaho.*¹—In the summer of 1943 mosaic was reported by the field inspectors of the Idaho Crop Improvement Association in a field of Great Northern U.I. 15 beans grown near Buhl, Idaho. As this variety is resistant to common bean mosaic the records were carefully checked and it became evident that a new type of mosaic was present in this field. Inoculation experiments in the greenhouse have fully established this fact.

Some of the Great Northern U.I. 15 seed used to plant the field mentioned was secured and planted in the greenhouse. Twenty-five plants were grown from this seed and 2 developed mottling and curling of the leaves somewhat characteristic of common mosaic. The symptoms were not so severe as those which developed in Red Kidney plants grown from seed known to be infected with common mosaic. One rather marked characteristic of the new mosaic seems to be a tendency for the tip of the leaflets to curl downward. This tendency is more marked than in common mosaic. Otherwise the symptoms

¹ Published with the approval of the Director as Research Paper No. 245 of the Idaho Agricultural Experiment Station.

of the 2 mosaics are similar and it is impossible to distinguish between them on the basis of symptoms alone. Seed was secured by E. C. Blodgett, of the Federal Emergency Plant Disease Prevention, from the field of beans grown near Buhl, Idaho, where the disease was first noted. This seed was planted in the greenhouse and of 37 plants only 7 developed mosaic symptoms. Since Dr. Blodgett reported 85 per cent infection in the field, this is a surprisingly small amount of seed transmission.

Seed was collected from Great Northern U.I. 15 plants grown in the greenhouse and infected with the new bean virus when in the seedling stage. These plants all exhibited mosaic symptoms. This seed was planted in the field and a record taken of diseased plants which were produced. Of 113 plants grown from this seed, 68 developed marked symptoms of the disease and 45 were apparently healthy.

Mosaic-free seed of a number of bean varieties was planted in the greenhouse and the plants were inoculated with the virus from Great Northern U.I. 15 plants infected with the new mosaic. A modification of the method described by Pierce² was used. Diseased tissue was ground in a small food chopper, the plant juice was extracted and applied with cheesecloth to the primary leaves of the seedlings before the trifoliate leaves had developed. Carborundum powder was dusted lightly over the leaves before they were rubbed with the cheesecloth saturated with the plant juice. The plants were grown under artificial light in order to induce the mosaic symptoms. Red Kidney plants were inoculated with common bean-mosaic virus at the same time to check the method of inoculation. Every plant inoculated with the common mosaic virus developed mosaic symptoms. Ten plants of Great Northern U.I. 15 were inoculated with common bean-mosaic virus and no symptoms appeared on any. Noninoculated checks were grown under similar conditions.

As a result of these experiments in the University of Idaho greenhouses at Moscow and in the greenhouses at the field station at Twin Falls, Idaho, it has been shown that 2 strains of the common mosaic virus are present in Idaho. The University of Idaho Great Northern selections Num. 1, 56, 59, 81 and 123 are all resistant to the new strain of the virus as well as to the common mosaic virus previously used in testing these selections for resistance. Great Northern U.I. 15, Red Mexican U.I. 3, and Red Mexican U.I. 34 which were obtained by crossing the Great Northern variety with the Red Mexican variety are susceptible to the new strain but resistant to the common mosaic virus. Several pinto-type segregants from crosses between the Red Mexican selections resistant to common mosaic and the common Pinto beans all proved to be susceptible to the new virus strain although resistant to the common mosaic virus. The Michelite, Robust, Red Kidney, Bountiful, and Burtner are all susceptible to the new strain of bean virus, while Idaho Refugee and U.S. No. 5 are resistant.

² Pierce, W. H. The identification of certain viruses affecting leguminous plants. *Jour. Agr. Res.* [U.S.] 51: 1017-1039. 1925.

Burkholder³ and Richards and Burkholder,⁴ reported a new virus disease of beans in New York and state that Norida, Red Mexican U.I. 3 and Great Northern U.I. 15 are all susceptible to it. They also reported Great Northern U.I. 1 and Great Northern U.I. 59 as being immune.

Several lots of bean seed of varieties used by Richards and Burkholder were secured from B. Lorin Richards and their reactions to the new virus found in Idaho were compared with reactions to the one reported in New York. The results indicate that the 2 viruses are identical so far as host reaction of the varieties tested is concerned.

During the field inspections of bean fields in southern Idaho by the officials of the Idaho Crop Improvement Association in 1944 and again in 1945, the new strain of bean mosaic virus was found only in one locality on Great Northern U.I. 15. None of the fields had more than one per cent of the disease and as no tolerance for mosaic is allowed, and a good supply of certified seed is available, it is hoped that the disease may be kept under control until a resistant substitute can be developed.

Due to the fact that the Great Northern selections are all resistant and many hybrids have been developed in the bean-improvement program, it is hoped that from these we may be able to secure satisfactory segregants which will combine resistance to the curly-top and the new mosaic viruses.—LESLIE L. DEAN, Plant Pathologist of the Idaho Leafhopper Fund Administration, and C. W. HUNGERFORD, Idaho Agricultural Experiment Station, Moscow, Idaho.

³ Burkholder, Walter H. New virus may threaten Robust pea-bean in New York. *Farm Research* 10: 12, 16. 1944.

⁴ Richards, B. Lorin, and Walter H. Burkholder. A new mosaic disease of beans. *Phytopath.* 33: 1215-1216. 1943.

ALBERT EDWARD EDGECOMBE

1897-1945

LEWIS HANFORD TIFFANY

The sudden death on March 30, 1945, of Professor Albert Edward Edgecombe at the age of forty-eight cut short the life of a man whose devotion to study and research had reached the stage where his accumulated knowledge and techniques would have resulted in maximal productivity. He was an Englishman by birth, spent over half of his life in Canada, and became an American citizen in 1935. He was an associate professor of botany at Northwestern University at the time of his death. His research activities were largely in the field of mycology.

Edgecombe was born in Devonshire, England, on February 5, 1897, and succumbed to a cerebral hemorrhage at Wilmette, Illinois, on March 30, 1945. He taught for eight years in the high schools of Canada and served for a time as a field agent of the Presbyterian Church in both Alberta and British Columbia. He entered Queen's University, Ontario, in 1921 and obtained the A.B. degree with honors in botany in 1923. In 1925 he was granted the M.A. degree from Queen's with a thesis on the rise of sap in woody plants. He was appointed to a graduate fellowship in botany at the University of Chicago in 1926 and received the Ph.D. from that institution in 1929. For his doctoral dissertation, prepared under the direction of Professor George K. K. Link, he investigated immunological relationships of certain wheats resistant and susceptible to rusts. He received the LL.B. and J.D. degrees from the Chicago Law School in 1934.

Dr. Edgecombe served as an assistant professor of botany at Northwestern University from 1929 to 1939, and as associate professor from 1939 until his death. He taught, at various times, general botany, plant genetics, mycology, plant pathology, and bacteriology. His special interests lay, however, in mycology and plant pathology, and recently he had made considerable study of certain dermatophytic fungi. He had worked on the mycetozoan flora of the Chicago Region for many years. His repeated attempts to culture certain of the obligate rusts on artificial media gave uniformly negative results. Post-doctoral studies in plant pathology, mycology, immunology, and medicine were made at Cornell University, University of Michigan, Pennsylvania State College, the Gradwohl Laboratories at St. Louis, and the Presbyterian Hospital of Columbia University.

Professor Edgecombe became a fellow of the American Association for the Advancement of Science in 1931 and was a charter member of the Mycological Society of America. In addition, he held membership in the American Phytopathological Society, Botanical Society of America, American Association of University Professors, Illinois Academy of Science, Sigma Xi, and Phi Alpha Delta. He married Sara Roberta Mohr of Pottstown, Penn-



ALBERT EDWARD EDGECOMBE
1897-1945

sylvania, on November 23, 1939. His widow and two children, Phyllis and David, together with one brother living in Canada, survive him.

Edgecombe was retiring, shy, and modest to the point of self-effacement. His qualities of friendliness, generosity, and affection were reserved for his family and his intimate friends. He loved the quiet of his home and the seclusion of his laboratory. He was an indefatigable worker, his interests ranged from poetry to medicine, and he died as he had wanted to live: quietly and with a minimum of disturbance to his fellow man.

Dr. Edgecombe was the author of the following publications:

- Further agglutination tests with phytopathogenic bacteria (with G. K. K. LINK and J. GODKIN). *Bot. Gaz.* **87**: 531-547. 1929.
Immunological relationships of wheats resistant and susceptible to *Puccinia rubigo-vera triticea*. *Bot. Gaz.* **91**: 1-21. 1931.
A comparative study of certain fungi. *Trans. Ill. State Acad. Sci.* **30**: 108-110. 1937.
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CERTAIN ASPECTS OF THE EPIPHYTOLOGY AND CONTROL OF TOMATO FRUIT ROT CAUSED BY PHYTOPHTHORA CAPSICI LEONIAN¹

W. A. KREUTZER AND L. R. BRYANT²

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INTRODUCTION

Since 1930 serious sporadic losses in certain truck crops in Colorado have been caused by *Phytophthora capsici* Leonian. This organism has been responsible for field losses in the State in peppers (*Capsicum annuum* L.) and eggplants (*Solanum melongena* L. var. *esculentum* Nees) (1), cucumber fruits (*Cucumis sativus* L.) (4), honeydew melon (*Cucumis melo* L. var. *inodorus* Naud.) and cantaloupe fruits (*Cucumis melo* L. var. *reticulatus* Naud.) (18), squash (*Cucurbita maxima* Duc.), watermelon vines (*Citrullus vulgaris* Schrad.) (5), watermelon fruits (19), and tomato fruits (*Lycopersicon esculentum* Mill.) (5). In addition, within the past two years the senior writer has isolated the organism from decaying fruits of banana squash (*Cucurbita maxima* Duc.) in northern Colorado fields.

Elsewhere in the United States, *Phytophthora capsici* has occurred in epiphytotic form in the field on pepper plants in New Mexico (9) and Florida (17), and in California on honeydew melon fruits (13). Fruit rot of tomatoes caused by both *P. capsici* and *P. drechsleri* was reported by Tompkins and Tucker (14) as occurring in California in 1937, 1938, and 1939. In this same publication a complete literature review on fruit rot of tomatoes and its causal agents is given. In addition, a root rot of pepper and pumpkin (*Cucurbita pepo* L.) caused by *P. capsici* has been described as occurring in the same State (15).

Fruit rot of tomatoes caused by *Phytophthora capsici* occurred in epiphytotic proportions in the Arkansas River Valley of Colorado in 1938 (5). In 1940 fruit rot of tomatoes occurred in northcentral Colorado in the Brighton area. Losses in each of the 1938 and 1940 epiphytotics in Colorado were estimated by canning companies to be approximately 50 per cent in those areas. In 1945, another outbreak of the disease in the Arkansas River Valley occurred. Losses were estimated at from 10 to 25 per cent.

Because of the high losses in these areas, a study was begun in 1942 to determine (a) the principal factors influencing the production of sporangia and swarmspores by the pathogen, (b) the effects of temperature and duration of exposure on infection of fruits by swarmspores, (c) the rôle of cultural practices or chemical treatments in the control of fruit rot in the field, and (d) the possibility of resistance in any commercial varieties, selections, hybrids, or types of tomatoes available.

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² The writers wish to express their appreciation to Mr. George H. Lane, Dr. A. O. Simonds, Mr. W. C. Hatfield, and Dr. L. W. Durrell, for assistance during certain phases of this study. They also wish to acknowledge the assistance of other members of the staff of the Colorado Agricultural Experiment Station in the course of the work.

EXPERIMENTAL RESULTS

Sporulation and Infection by Phytophthora capsici

From both field observations and experimental work, it is indicated that epiphytotic of tomato fruit rot caused by *Phytophthora capsici* depend primarily on sporangium and swarmspore formation (7). Accordingly, before control measures could be developed and evaluated it seemed necessary to determine the conditions under which the causal organism would produce sporangia and swarmspores, and the factors influencing fruit infection by swarmspores.

In this study two isolates were used. Culture 5C was obtained from rotting tomato fruits in northcentral Colorado, and culture 6C was obtained from fruits growing in southeastern Colorado. These isolates were morphologically similar to other previously identified cultures obtained in the State (5, 6). All isolates of the organism have been virulently pathogenic to non-wounded pepper plants (*Capsicum annuum* L.). With the exception that local isolates either form no sexual fruiting bodies or produce them only after 6 to 8 months in culture, they conform with the adequate descriptions of *Phytophthora capsici* (9, 13, 15, 16, 19).

Formation of Sporangia and Swarmspores.—In previous studies by the writers (7), local isolates readily produced abundant sporangia when non-steamed field soil was used as a medium for their development. The optimum temperature range for the growth of certain isolates of *Phytophthora capsici* has been given by Godoy (3) as 24° to 26° C. Tompkins and Tucker (13) found the optimum growth range for one isolate to be 25° to 30° C. Wiant and Tucker (19) reported the optimum temperature range for growth of another isolate to be 26.7° to 29.4° C. Kreutzer *et al.* (6) found that the optimum temperature for sporangium production of certain isolates of *P. capsici* obtained from wilted pepper plants and rotted cucumber fruits was near 30° C.

To determine the optimum temperature for sporangium production using nonsteamed field soil as a medium, giant cultures on barley were ground and mixed with approximately five times as much nonsteamed field soil by volume. The soil was treated as in previous studies (7) except that the moistened soil-inoculum mixture was placed in clay pots and incubated at 10°, 15°, 20°, 25°, 30°, and 35° C. at 100 per cent relative humidity for from 48 to 64 hours. The determination of the presence and relative quantity of sporangia was made using a previously described technique (7), placing one cubic inch of the soil mixture in a 400-ml. beaker containing approximately 30 ml. of tap water, allowing this mixture to stand at 25° C. for from 2 to 4 hours, and then examining drops for the presence and relative numbers of swarmspores at hourly intervals within that period.

Sporangia were produced only at temperatures ranging from 20° to 30° C. inclusive, the greatest quantity being produced at 25° C. No sporangia were produced at 10°, 15°, or 35° C. In other similar trials results were

essentially the same. Another important point was brought out in subsequent tests; while sporangia were produced at 20° C., none were formed at a controlled temperature of 18° C.

The 2- to 4-hour period used to determine the relative numbers of swarmspores produced was the result of trials which, in general, showed that at

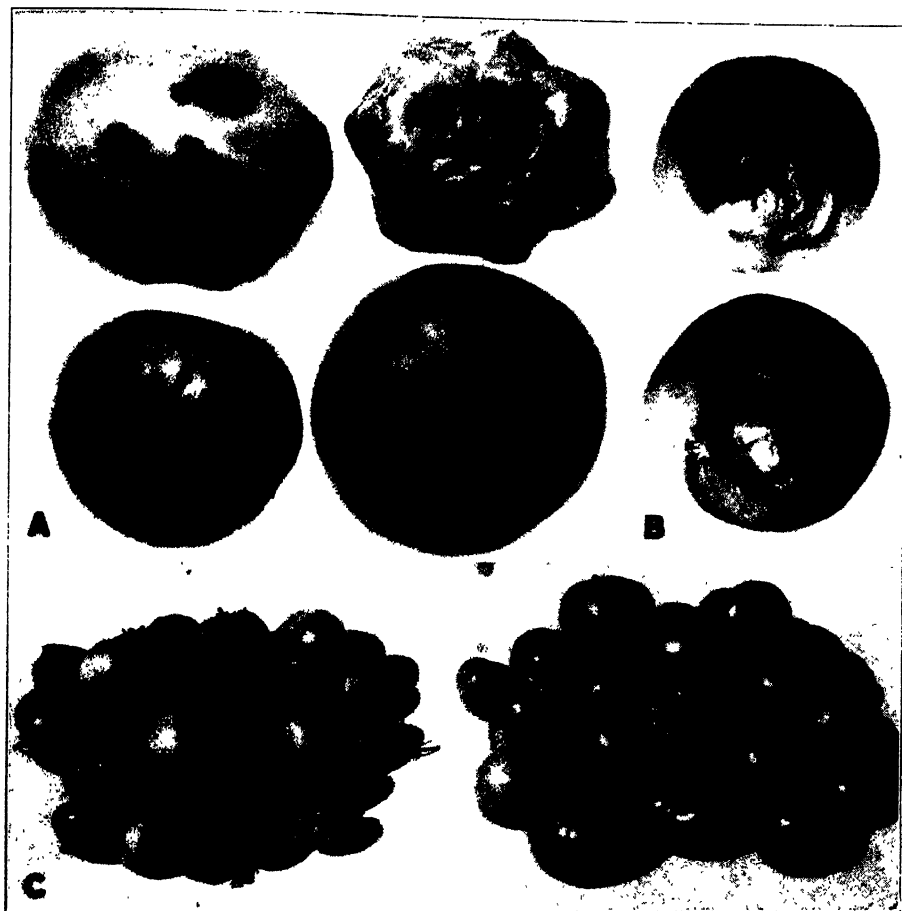


FIG. 1. Fruit rot of tomatoes caused by *Phytophthora capsici*. A. Technique used to test infectivity of *P. capsici* under varying conditions. Lower right: drops containing swarmspores on uninjured fruit surface. The other three fruits show progressive stages in infection at 3-day, 6-day, and 14-day intervals at room temperatures. B. Two types of fruit-rot symptoms. C. The yield of a single plant showing loss caused by the disease. Left, fruits which were in contact with the soil. Right, fruits which were not in contact with the soil.

25° C. only a few swarmspores were released in 90 minutes, while after 150 minutes swarmspores were abundant. In most cases swarmspore production was apparent in from 30 to 60 minutes and reached its peak in from 2 to 3 hours.

Swarmspore Germination and Infection.—The rôle of temperature and time in the germination of and infection by swarmspores was determined.

In one study the effect of time exposure on infection at varying temperatures was investigated. Green fruits³ of the Landreth variety were inoculated by placing two to three drops of inoculum containing numerous active swarmspores on the styler ends (Fig. 1, A). They were then incubated at 20°, 25°, and 30° C. at 100 per cent relative humidity for 40, 60, 80, 100, 120, 140, 160, and 180 minutes. Ten inoculated fruits were used for each time exposure at each given temperature. At the end of each time interval, fruits were removed from the incubators and the drops of inoculum were removed by gentle blotting with clean cheesecloths. These fruits were then placed on laboratory tables at room temperatures. Fruits were examined at 12-hour intervals for 6 days. At the end of this period final readings were made. In figure 1, A are typical reactions to inoculation by use of this technique. The results of this study, with isolates 5C and 6C, are in table 1.

TABLE 1.—*Effect of time of exposure at varying temperatures on infection of tomato fruit (variety Landreth) by Phytophthora capsici*

Exposure (minutes)	Percentage of inoculated areas infected at		
	20° C.	25° C.	30° C.
40	0.0	0.0	0.0
60	0.0	10.0	15.0
80	10.0	35.0	45.0
100	40.0	85.0	65.0
120	70.0	85.0	85.0
140	75.0	90.0	80.0
160	60.0	60.0	90.0
180	90.0	100.0	100.0

The earliest infection observed occurred after exposure for 60 minutes at 25° and 30° C. No infection was noted at 20° C. until exposure was increased to 80 minutes. Infection at 20° C. was consistently less than at 25° C. and 30° C. except after the longer exposures. Temperatures of 25° C. and 30° C. were equally effective at all exposure periods tested.

In a number of parallel tests the results were similar to those given in table 1. In no instance was infection observed after exposure of less than 60 minutes at any temperature and in no case was infection observed at less than 80 minutes exposure at 20° C.

In other studies, exposures of fruit to swarmspore inoculum at 10° C. brought about infection. In these tests fruits were exposed to drops of inoculum for 5 hours at 10°, 15°, and 20° C. All fruits were infected. No attempt was made to determine the shortest exposure time for infection at 10° C.

In another trial a total of 910 green fruits of uniform size from 40 varieties, lines, and types of tomatoes were used. The fruits were first randomized on clean laboratory tables and held for 24 hours at room temperatures to equalize all fruit temperatures. They were then inoculated by placing

³ Green fruits were used in all tests rather than red or ripening fruits, since infection in its earliest stages was more readily detected on green fruits.

3 drops of water containing active swarmspores of culture 6C on the stylar end without injury to the fruit. The drops were allowed to dry naturally, and the drying time was recorded for individual fruits. Drying intervals fell within a range of 40 to 170 minutes. The fruits were placed in classes of 20-minute intervals, giving 6 class intervals of 40-59, 60-79, 80-99, 100-119, 120-139, and 140-169 minutes. During the trials, thermometers were placed in check fruits at random on the tables to obtain temperature variations. A recording of infections was made 10 days after the time of inoculation. The results are in table 2.

The drops on the majority of fruits (506 cases) dried in from 60 to 99 minutes. The earliest infection, together with the smallest number of infected fruits (5.0 per cent), was observed in the 60-79-minute interval. The greatest amount of infection (72.7 per cent) appeared in the 140-169-minute interval.

TABLE 2.—*Effect of time of exposure on infection of tomato fruits by Phytophthora capsici at room temperature (22-24° C.)*

Time interval (minutes)	Total number of fruits inoculated	Percentage of fruits infected
40-59	58	0.0
60-79	261	5.0
80-99	245	23.7
100-119	191	48.1
120-139	111	57.6
140-169	44	72.7

Another series of studies was designed primarily to determine the degree of swarmspore activity and compare it with infection at varying time intervals. Temperatures were held uniform at 25° C. and the same techniques were used as in previous tests except that the drops of swarmspore-inoculum on fruits were washed off with 95 per cent ethyl alcohol after each lot of fruit was removed from the incubator at a given time interval. In addition, to determine swarmspore activity and germination at each time interval, several inverted drops of swarmspore inoculum on slides mounted on glass blocks in Petri dishes were placed in the incubator for each inoculated lot of fruits. The drops were examined for swarmspore activity and germination when each lot of fruits was removed from the incubator. The results of this study are in table 3.

Quiescent swarmspores were first noted at 80 minutes, and the earliest germination occurred in 100 minutes. This correlates perfectly with the shortest time at which infection was observed. At 140 minutes, a few swarmspores still were active, but at 160 minutes all activity had ceased. Inoculated fruits were washed with alcohol at the end of each exposure interval and blotted as in previous tests. This disinfectant undoubtedly destroyed all spores on the fruit surface and all germ tubes which were not established within the cuticle. This may explain the comparatively long time interval required for infection (100 minutes) as against a shorter period of 80 minutes for previous tests.

TABLE 3.—*Effect of time of exposure at 25° C. on swarmspore activity and germination, and infection of Landreth tomato fruit by swarmspores*

Exposure (minutes)	Observations on swarmspores	Infection of inoculated fruits
20	All swarmspores very active.	—
40	All swarmspores active.	—
60	Swarmspores less active.	—
80	A few swarmspores quiescent, majority still active.	—
100	Majority of swarmspores quiescent, a few germinating.	+
120	Majority of swarmspores germinating.	+
140	Almost all swarmspores quiescent, germination general.	+
160	All swarmspores quiescent, germination marked.	+
180	Almost all swarmspores germinating.	+

Control Investigations

Since sporangia apparently are not formed at 18° C. (64.4° F.) or below, an epiphytotic of fruit rot should not occur at such temperatures even though abundant moisture is present. Observations in commercial tomato fields in canning districts support this view. However, with favorable temperatures for sporangium production (above 20° C.) and abundant free water, sporangia will form rapidly within 24 to 48 hours, and swarmspores will be released in from 1 to 3 hours. Following the release of swarmspores infection can occur at any field temperature, and at favorable temperatures of 25° to 30° C. can occur in as short a time as 60 minutes. These findings emphasize that for effective prevention of tomato-fruit rot, control measures should be applied before conditions favor an epiphytotic.

Use of Cultural Methods.—In 1942 a study was conducted to determine if mulching the bases of plants with straw would aid in reducing infection caused by *Phytophthora capsici*. In two 160-foot rows of a plot in which the soil was inoculated by the method described in an earlier paper (7), plants of both Landreth and Early Baltimore varieties were straw-mulched. Control plants left in small five-plant blocks were not mulched. Records were taken on 1,827 fruits, from 30 plants. The results are in table 4.

Although the design of the 1942 test was such that the data obtained were unsuited for statistical analysis, the wide differences in results between the mulched plants (93.8 and 83.5 per cent healthy fruits) and the nonmulched plants (42.2 and 29.5 per cent healthy fruits) indicate that mulching reduced fruit rot to a marked degree.

TABLE 4.—*Effect of straw mulching on field infection of fruits of the Landreth and Early-Baltimore varieties of tomato*

Variety	Percentage of healthy tomato fruits from	
	Mulched plants	Nonmulched plants
Early Baltimore	93.8	42.2
Landreth	83.5	29.5
Total	88.6	36.0

In 1943 staking and low ridging (6 inches) were included with mulching in another field experiment. Staking tomato plants was recommended by Sherbakoff (11) as a possible control for Buckeye rot of tomato caused by *Phytophthora terrestris* (*P. parasitica*). Sherbakoff, however, had no experimental staking trials. In the test conducted by the writers, 120 plants of the Landreth variety were set out in a randomized block of 12 plots. Each treatment was replicated three times. The soil was inoculated by the soil-inoculum method (7) and irrigated at frequent intervals. At harvest, all plants were stripped of fruit. Readings were made on 5,509 individual fruits. The results are in table 5.

Ridging resulted in significantly⁴ more healthy fruits than the control. In mulched and staked plots, the increases in healthy fruits over the control were highly significant, and an increase in yield of healthy fruits approach-

TABLE 5.—Effect of cultural treatments for the reduction of tomato fruit rot. Results are given as three-plot averages of healthy fruits harvested

Treatment	Average percentage of healthy fruits	
	Actual	Transformed ^a
Control	60.53	51.12
Ridging	80.78	65.00
Mulching	89.42	71.13
Staking	98.05	82.46
Difference required for significance 5 per cent level = 12.09		
Difference required for significance 1 per cent level = 18.31		

^a Percentages converted into $\sin^2 \theta$ by use of Bliss's tables (2, 8).

ing the 1 per cent level of significance was obtained in staked plots when compared with that obtained by ridging.

Use of Dust Fungicides.—Because fruit rot appears in commercial tomato fields late in the season, the large sprawling vines make spraying or dusting with power equipment an impractical undertaking, and few growers or canning companies are interested in hand spraying. Dusting by hand, however, is considered practical and is frequently employed in Colorado late in the season to control certain insects. For these reasons, the effectiveness of various fungicidal dusts in controlling tomato fruit rot was determined.

As a preliminary to field trials, an investigation was conducted in the laboratory using fruits grown in the greenhouse. A method was devised to give a rough approximation of the fungicidal value of any dust. Fruits to be tested were individually mounted on watchglasses and then were given a light dusting with a fungicide. Following this they were inoculated by sprinkling lightly with drops of water containing motile swarmspores of *Phytophthora capsici*. The inoculated fruits were placed in incubators at 25° C. at 100 per cent humidity or in moist chambers at room temperatures

⁴ Where "significant" is used, it refers to the 5 per cent level of significance. "Highly significant" refers to the 1 per cent level of significance.

for from 2 to 4 hours. At the end of this time the fruits were removed and the drops dried by blotting. The fruits were then held on laboratory tables to determine whether infection would develop.

The most effective fungicides used were those which contained copper as the active principal. Dusts selected for field testing were 5 and 10 per cent Yellow Cuprocide, 10 per cent Basicop, 10 per cent tribasic copper sulphate, and 10 per cent copper oxychloride. Bordeaux spray (4-4-50) was included in the test as a fungicidal standard. To test the efficacy of these chemicals in the field, a planting of tomato plants of the Landreth variety was divided into three blocks, each containing eight randomized plots (Table 6). The treatments were next applied and the soil of the plots was inoculated using the soil-inoculum technique (7). Frequent irrigations and the occasional use of a system of overhead sprinklers kept the field wet for 10 days. At the end of this time the treatments were again applied to the plots and were followed by a light sprinkling. The planting consisted of 24 plots containing 240 plants. The fruits on 238 plants were counted and read for disease. Fruits were divided on the basis of whether they were in contact with the ground or above the ground (Fig. 1, C). Fruits resting on fruits in contact with the ground were considered in the ground-contact group. A total of 11,385 fruits were recorded on the basis of health or rot; 5,348 of these were in contact with the ground and 6,037 were above the ground. Readings were made 30 days after the inoculation. The results are in table 6.

Analysis of transformed data (2, 8) given in table 6 indicates that where fruits were in contact with the ground, all treatments except Nos. 4 (10 per cent Basicop) and 5 (10 per cent tribasic copper sulphate) gave a highly significant control when compared with the untreated check (No. 8). The percentage differences between treatments 4 and 5 and the control were nonsignificant.

Where only fruits above the ground are considered, one treatment, No. 2 (5 per cent Yellow Cuprocide talc), gave significantly more healthy fruit than the control.

If fruit totals are considered, irrespective of position on the plant, all treatments except Nos. 4 (10 per cent Basicop), 5 (10 per cent tribasic copper sulphate), and 3 (10 per cent Yellow Cuprocide), gave highly significant increases in healthy fruit over the control. Treatment No. 3 (10 per cent Yellow Cuprocide) was significantly better than the control. The differences between treatments 4 and 5 and the control were not significant.

Use of Copper Sulphate in Irrigation Water.—Muller (10), who made his observations in the Netherlands East Indies on a foot rot of black pepper (*Piper nigrum* L.), recommended the use of a network of shallow drain trenches to prevent infection by *Phytophthora palmivora* var. *piperis*. When infection occurred in one of the squares isolated by trenches, Muller recommended that the diseased plants be watered with 5 to 10 liters of a 1 per cent solution of copper sulphate per square meter to prevent spread of the disease.

TABLE 6.—*Effect of chemical treatments for the control of tomato fruit rot on the Landreth variety, 1943. Results are given as three-plot averages of percentages of healthy fruits*

No.	Treatment	Average percentages of healthy fruit					
		On ground		Above ground		Total	
		Actual	Trans- formed ^a	Actual	Trans- formed	Actual	Trans- formed
1	5 per cent Yellow Cupro- cide, 95 per cent Cherokee clay, dust	60.71	51.43	98.58	83.96	82.10	65.30
2	5 per cent Yellow Cupro- cide, 95 per cent tale, dust	65.48	54.32	99.14	85.65	83.60	66.26
3	10 per cent Yellow Cupro- cide, 90 per cent tale, dust	59.11	50.28	97.60	81.55	77.82	62.00
4	10 per cent Basicop, 90 per cent tale, dust	48.04	43.87	97.48	81.01	72.69	58.57
5	10 per cent tribasic copper sulphate, 90 per cent tale, dust	42.67	40.73	98.11	82.45	71.93	58.17
6	10 per cent copper oxy- chloride, 90 per cent tale, dust	63.59	52.97	98.36	82.69	82.37	65.38
7	4-4-50 Bordeaux mix- ture, spray	70.38	57.15	98.52	83.48	83.17	66.02
8	Control, no treatment	32.03	34.18	95.37	78.71	65.43	54.05
Difference required for significance:							
5 per cent level			11.32			6.23	7.74
1 per cent level			15.71			8.64	10.74

^a Actual percentages transformed to values of p as $\sin^2 \theta$ by use of Bliss's tables (2, 8).

In a preliminary trial in 1944, to determine the value of CuSO_4 in irrigation water as a control for *Phytophthora capsici*, a small plot was planted with tomatoes of the Landreth variety. Inoculation of the soil was begun during cool weather (September 7) since fruit development had been very late. A total of eight 135-foot rows were used in the study. Each of four rows was treated with $5\frac{1}{2}$ pounds of CuSO_4 and four rows were held

TABLE 7.—*Effect of the addition of copper sulphate to irrigation water for the control of tomato fruit rot. Results are given as four-plot averages of healthy fruits harvested*

Treatment of irrigation water	Average percentage of healthy fruits	
	Actual	Transformed
CuSO_4 added to irrigation water at rate of approximately 4 pounds per 100 feet of row	97.46	81.42
Control, no CuSO_4 added to irrigation water	89.94	71.73
Diff. required for significance at 5 per cent level		5.61
Diff. required for significance at 1 per cent level		9.30

as controls. Copper sulphate treated and control rows were separated by two-row buffers. The fruit on the first 10 plants of each row were harvested and read for disease. A total of 80 plants bearing 11,040 fruits were read. The results are in table 7.

The data show a highly significant increase in the average percentage of healthy fruits harvested in favor of the CuSO_4 -treated lots. The actual average percentage of healthy fruit harvested for the control plots, 89.94, is a very high figure, indicating only light infection.

Resistance to Tomato Fruit Rot.—Of all the commercial varieties of tomatoes tested to date for resistance to tomato fruit rot, none have shown any consistently resistant reactions. In 1942, 120 commercial varieties, lines, and types were tested in the field in small blocks. Only a few of these lines showed any promise. Although not very consistent in its reactions, the variety Porter apparently has some resistance.

The fruits of all plants being tested are subjected to a laboratory test. All fruits are inoculated by placing drops of water containing swarmspores on their noninjured styler ends. The styler end is subjected to infection because fruits generally appear to be more resistant to infection at the calyx end (12). Fruits are then incubated and treated as previously described. Only those having definitely arrested lesions are considered resistant. These studies have not progressed to the point where any definite statements regarding resistance can be made.

SUMMARY

Cultures of *Phytophthora capsici* isolated from rotting tomato fruits formed sporangia in great abundance within 48 hours at 25° C., when 20- to 60-day-old giant barley cultures were ground and mixed with well-moistened, nonsteamed field soil.

The optimum temperature for sporangium development by these isolates was about 25° C. Sporangia were not produced at 18° C. or lower temperatures, but occurred readily between 20° and 30° C. Sporangia were not produced at 35° C. Although abundant moisture is an essential factor, the prime requisite for an epiphytotic is a soil temperature between 65° and 85° F.

When water was added to soil-inoculum that had been held at 25° C. for 48 hours swarmspore production reached its peak in from 2 to 3 hours at 25° C.

Earliest infection of noninjured green tomato fruits occurred after 60-minute exposure to drops of inoculum containing swarmspores, at 25° and 30° C. Infection reached its peak after 180-minute exposure at 25° and 30° C. Exposure of fruits to swarmspores at 10° C. for 5 hours resulted in infection. Studies of swarmspore activity showed that the time required to produce infection was correlated with the time required for swarmspore germination.

The rapidity of sporangium formation at optimum temperatures and the

release and germination of swarmspores indicate that control measures must be applied early if they are to be effective.

Tests on cultural practices for the control of fruit rot showed that mulching with straw or staking resulted in more healthy fruit than corresponding controls (1 per cent level of significance). Ridging produced significantly more healthy fruit than the controls (5 per cent level of significance).

Studies on the use of fungicidal dusts for the control of fruit rot showed that where fruits in contact with the ground were considered, 5 per cent and 10 per cent cuprous oxide and 10 per cent copper oxychloride gave highly significant (1 per cent level) control. Bordeaux spray 4-4-50 also effectively controlled the disease.

Preliminary studies on the addition of CuSO_4 to irrigation water at the time of soil inoculation, indicated a highly significant degree of control.

Of 120 commercial varieties, lines, and types of tomato tested in the field, a few lines have shown slight promise in resistance studies.

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HOST-PARASITE RELATIONSHIPS OF THE ROOT-KNOT NEMATODE, *Heterodera marioni* (Cornu) Goodey, II. SOME EFFECTS OF THE HOST ON THE PARASITE¹

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The feasibility of growing a particular crop on land infested with the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, depends, in most instances, on two factors: (a) The extent to which the crop in question will be injured and yield thereby reduced and (b) the amount of residual soil infestation that will carry over to infect the following crop. The first factor will be determined, primarily, by the effects of the parasite on the host and the second factor may be determined, to a considerable extent, by the effects of the host on the parasite. These effects of the host on the parasite become manifest in various ways, *e.g.*, by the rate at which the nematodes develop, by the extent to which they develop, by the percentage of females that reach maturity, and by the number of eggs laid by these females. However, these are not distinct and independent manifestations but are, to some extent, interdependent.

Development of the root-knot nematode is influenced, of course, by environmental factors other than the host, of which temperature seems to be the most important. Demonstrating the effect of this factor, Tyler (5) found that in tomato, grown in soil at 27° C., 16 to 19 days elapsed from the time larvae entered the roots until females began to lay eggs. When the plants were grown in soil at 24° C., this interval was 25 to 27 days, and at 15.5° C. it was 67 days. Demonstrating the effects of the host on parasite development, Godfrey and Oliveira (3) found that in cowpea, grown at a temperature permitting rapid development, the interval between penetration of larvae and first deposition of eggs was 19 days, but that in pineapple, grown under identical conditions, it was 35 days.

Using population 8 of the root-knot nematode (see Table 1) and growing the plants in a greenhouse where the temperature averaged slightly below optimum for rapid development of the parasite, the writer found that in common tobacco (*Nicotiana tabacum* L.) about 20 days usually elapsed from the time larvae entered the roots until females began to lay eggs. In turnip (*Brassica rapa* L.), grown under identical conditions, this interval was increased by 8 or 10 days but most of the females eventually reached maturity and egg output was not noticeably less than in common tobacco. In *Nicotiana megalosiphon* Heurck & Muell. Arg. development was much slower than in turnip, only a few females (probably less than 1 in 50) ever reached maturity and those that did laid much fewer eggs than in common tobacco. In *N. plumbaginifolia* Viv. development was retarded more than in *N. megalosiphon* and only a few parasites ever passed the molting stage.

¹ For the first paper of this series see literature cited (2).

A meticulous examination of the entire root system shown in figure 2 revealed only one egg-laying female, a translucent, ill-nourished individual about one-third normal size, that had deposited 7 eggs. In *Crotalaria spectabilis* Roth. development progressed even slower than in *N. plumbaginifolia* and no parasite was seen that had passed the molting stage. Larvae entered the roots of all these plants freely but with *C. spectabilis* moderately swollen root tips were about the only macroscopic evidence of infection. With *N. megalosiphon* and *N. plumbaginifolia*, on the other hand, galling was only slightly less conspicuous than with common tobacco (Fig. 2).² These differences in parasite development resulted from interactions between the same race of nematode and different host plants but it eventually became obvious that equally pronounced differences may result from interactions between different races of the parasite and the same host plant.

Reported in this paper are the results of several experiments to ascertain the effects of the host on the parasite, especially on rate and extent of development. Supplementing these experiments are the results of certain observations, demonstrating, to some extent, how these effects of the host on the parasite may influence the building up and maintenance of nematode populations.

PROCEDURE

One object of the procedure employed was to control or equalize, so far as possible, factors other than host that influence the development of the parasite. This was accomplished, at least to some degree, by so arranging the experiments that the various dates when the plants representing one host-parasite combination were infected corresponded approximately with the dates when the plants representing another host-parasite combination were infected; by growing the plants simultaneously in the greenhouse; and by securing data on a representative sample of the parasites harbored by each plant. Air temperatures in the greenhouse were 18° to 21° C. during the night and 24° to 27° C. during the day.

Nematode populations used. In the previous paper of this series (2) the term population was used to designate "an aggregate of individuals of the root-knot nematode the progenitors of which were secured from a single plant or from several plants of the same kind that grew together in the same locality." This use of the term is continued in the present paper. The manner by which the nematodes were collected does not preclude the possibility that a population includes more than one race but considerable work with these populations has failed to produce any very convincing evidence that such is the case. However, methods for identifying and differentiating races have not been perfected. The differences between some races are very pronounced and easy to demonstrate but to differentiate every

² The ability of population 8 to produce galls on *Nicotiana plumbaginifolia* may be somewhat unusual since the writer has learned through personal communication with Dr. E. E. Clayton, Division of Tobacco, Medicinal, and Special Crops, that this plant has been grown in heavily infested soil of several localities in the southeastern states yet appreciable galling has never been observed.

race from every other race is a task that will be neither easy nor quickly accomplished. The designations and origins of the populations used in the following experiments or referred to in the discussion are given in table 1. It should not be assumed that the plant from which a particular race was collected is an especially suitable host for this race or that the plant will be found more commonly infected by this race than by some other. The writer has had under investigation 6 populations from potato (*Solanum tuberosum* L.) each collected in a different locality. These 6 populations included 5 different races. Potatoes become infected with whatever race happens to occur in the region where they are grown and undoubtedly the same is true of many other plants. For example, there is no reason to assume that population 6, by virtue of the fact that it was collected from sweet potato (*Ipomoea batatas* (L.) Lam.), occurs more commonly on this crop than population 8. Whether this is or is not the case depends on which population happens to be more widely distributed in the sweet-potato-growing regions.

TABLE 1.—*Sources and designations of the root-knot nematode populations*

Pop. No.	Original host	General locality where collected
1A	Alfalfa	Shafter, Calif.
2	Cotton	Shafter, Calif.
3B	Potato	Harlingen, Tex.
3C	do	Tampa, Fla.
3D	do	Long Island, N. Y.
4	Peanut	Pitt County, N. C.
5	Parsnip	Falls Church, Va.
6	Sweet potato	Beltsville, Md.
8	Tomato	Greenhouse, Beltsville, Md.

Inoculation of plants. Soil and pots used for growing the plants were autoclaved 30 to 40 min. at 15 lb. pressure. Small healthy plants, secured by sowing seed or by some suitable method of vegetative propagation, were set into thumb pots. Each plant was allowed to grow until a loose network of roots had formed over the inner surface of the pot and the plant could be removed with the root system and soil mass intact. With the aid of a pipette the inoculum (a suspension of viable larvae in water) was applied to the sides and bottom of the soil mass and the plant returned to the pot. After 24 hours the plant was again removed and all the soil washed away from the roots. Washing was done carefully to avoid injuring the roots, but thoroughly to remove all larvae that had not already penetrated. The plant was then reset in a larger pot, watered, and shaded from bright sunlight for the first day or two. When the roots of the plant were subsequently examined the age of the parasites, *i.e.*, the length of time they had been in the roots, was known to within the limits of 24 hours.

Examination of plants. Each plant was removed from the pot, the soil washed from the roots, and the entire root system treated with lacto-phenol. The technic was essentially the same as that described by McBeth, Taylor,

and Smith (4) except that Sudan III replaced acid fuchsin as a stain. This treatment clears and softens the roots and stains the parasites, making them easy to see and to remove. A small portion of the root system was clipped off and examined under a low-power, dissecting microscope. So far as possible every parasite contained in this portion of the root system was dissected out and its stage of development determined and recorded. Another portion of the root system was then examined and the operation continued until 100 parasites had been counted and recorded or until the entire root system had been examined. One plant was used for each count.

Data recorded. The parasites were classified into 5 groups based on the amount of development undergone. Group A includes larvae from the

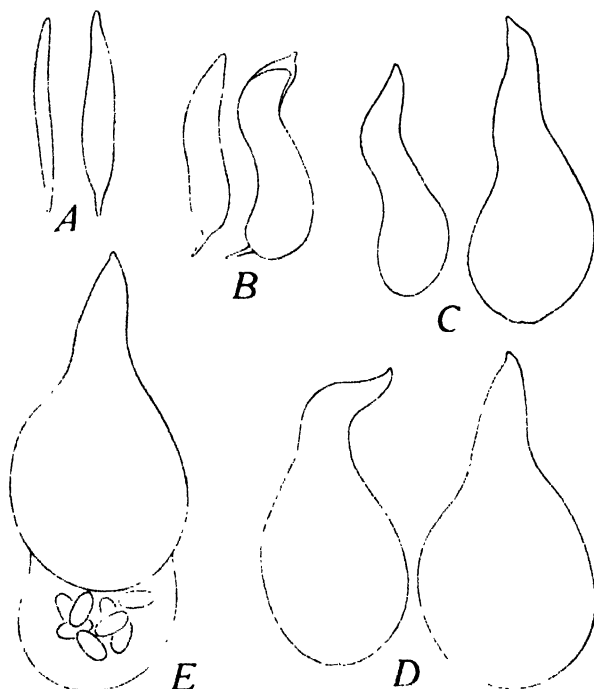


FIG. 1. Groups A through E into which the parasites were classified according to the amount of development undergone.

stage where they have begun to grow to the stage where they still possess a more or less conical tail (Fig. 1, A). Group B includes larvae from the stage where they have acquired a more or less hemispherical posterior end, terminated by a spike, to the stage where they are about to complete the final molts (Fig. 1, B). Group C includes females from the stage where they have completed the molts to the stage where they are almost, though obviously not quite, fully grown (Fig. 1, C). Group D includes females that are fully grown or almost fully grown but have not yet laid eggs (Fig. 1, D). Group E includes egg-laying females (Fig. 1, E).

Because the separation of group A from group B and of group C from

group D depends on artificial cleavages in a graded series, some inconsistencies in placing borderline individuals were unavoidable but the error thus introduced is not sufficient to alter the final picture materially. Sudan III, as used in the technic, does not stain larvae that have undergone no development and if such occurred they are not included in the counts. Adult males were omitted; metamorphosing males were placed in group B.

In the first experiment (Table 2) only 50 parasites from each plant were recorded and no distinction was made between fully-grown females that had, and those that had not, laid eggs. It became obvious, however, that

TABLE 2.—*Comparative development of the root-knot nematode (population 8) in certain varieties of sweet potatoes*

Plant	Period of development		Number of parasites in each group				
	Date infected	Time	A	B	C	D&E	Total
<i>Ipomoea batatas</i> (L.) Lam., varieties							
Triumph	Aug. 4-5	3	9	27	12	2	50
		5	6	17	12	15	50
Porto Rico	do	3	10	23	14	3	50
		5	3	6	7	34	50
Nancy Gold	do	3	7	16	17	10	50
		5	0	1	3	46	50
Red Brazil	do	3	0	5	9	36	50
		5	0	0	0	50	50
Jersey Big Stem	Aug. 5-6	3	0	3	16	31	50
		5	6	2	7	41	50
Florida	do	3	3	7	13	27	50
		5	0	0	3	47	50
U.S. No. 47442	do	3	2	9	12	27	50
		5	0	2	7	41	50
Orange Little Stem	Aug. 6-7	3	3	3	0	0	6
		5	0	4	4	0	8
Nancy Hall	do	3	2	9	18	21	50
		5	0	7	3	40	50
Mameyita	Aug. 11-12	3	2	8	33	7	50
		5	0	22	21	7	50

50 was not an adequate number of parasites and that the data would be more informative if egg-laying females were placed in a separate group.

Short time intervals were used in the first experiment lest results be invalidated by the inclusion of second-generation larvae. In subsequent experiments 6-week and, finally, 8-week intervals were included. That second-generation parasites were not encountered is apparent from an examination of the data.

EXPERIMENTAL RESULTS

Results are shown in tables 2 to 7. In the experiment with sweet potatoes (Table 2), parasite development of population 8 was most rapid in Red Brazil though only slightly more so than in Nancy Gold, Jersey Big Stem, Florida, U.S. No. 47442, and Nancy Hall. Development was retarded in

Mameyita and, to a less degree, in Triumph. Orange Little Stem acquired few parasites but the condition of those present suggested that development was somewhat retarded in this variety also.

Reference has already been made to the different rates at which population 8 developed when common tobacco, turnip, *Nicotiana megalosiphon*, and *N. plumbaginifolia*, served as hosts. These differences are clearly indicated by the data in table 3. When development stops, the parasite soon becomes translucent and, eventually, can no longer be recognized, which is probably

TABLE 3.—Comparative development of the root-knot nematode (populations 1, 1, 2, 4, and 8) in certain "susceptible" and in certain "resistant" plants

Plant	Pop. No.	Period of development		Number of parasites in each group						
		Date infected	Time	A	B	C	D	E	Total	
		1942-43	weeks							
<i>Nicotiana tabacum</i> L. var. Gold Dollar	S	Aug. 18-19	4	0	4	13	63	20	100	
	S	Aug. 31-Sept. 1	4	0	4	29	48	19	100	
<i>Brassica rapa</i> L. var. Purple-top Strap-leaf	S	Aug. 18-19	4	0	13	65	22	0	100	
	S	do	6	0	2	11	26	61	100	
<i>Nicotiana megalosiphon</i> Heurek & Muell. Arg.	S	Aug. 24-25	4	13	70	17	0	0	100	
	S	do	6	2	59	33	5	1	100	
	S	Sept. 2-3	8	1	24	7	3	0	35	
<i>Nicotiana plumbaginifolia</i> Viv.	S	Aug. 24-25	4	65	1	0	0	0	66	
	S	do	6	0	0	0	0	0	0	
<i>Lantana camara</i> L.	S	Aug. 31-Sept. 1	4	0	0	0	0	0	0	
<i>Pelargonium graveolens</i> L'Her.	1A	Jan. 9-10	6	3	0	0	0	0	2	
	2	do	6	0	0	0	0	0	0	
	4	Jan. 1-2	6	0	1	2	14	0	17	
	S	do	6	0	0	0	0	0	0	
<i>Senecio cineraria</i> DC.	1A	Jan. 18-19	6	15	46	33	6	0	100	
	2	do	6	0	3	2	2	2	9	
<i>Arachis hypogaea</i> L. var. Virginia Runner	1A	Feb. 1-2	6	15	45	0	0	0	60	
	2	do	6	6	16	0	0	0	22	
	4	do	6	0	1	3	9	87	100	
	6	do	6	0	0	0	0	0	0	

the reason why no parasite was found in the 6-week plant of *N. plumbaginifolia* and is possibly the reason why so few were found in the 8-week plant of *N. megalosiphon*. That the same host may affect the development of different races of the parasite in a different manner is shown by the results, with peanut (Table 3). (See also results with cotton, *Gossypium hirsutum*, and alfalfa, *Medicago sativa* L., in tables 6 and 7.)

Population 8 developed at about the same rate in each of the varieties of bean (*Phaseolus vulgaris* L.) tested (Table 4) with the probable exception of Alabama No. 1 on which, unfortunately, results are incomplete.

The average rate of development in *Taraxacum kok-saghyz* Rod. was about the same for each of the populations used (Table 5) but with 2, 5.

TABLE 4.—Comparative development of the root-knot nematode (population 8) in certain varieties of beans

Plant	Period of development		Number of parasites in each group						
	Date infected	Time	A	B	C	D	E	Total	
	1942-43	weeks							
<i>Phaseolus vulgaris</i> L., varieties Stringless Green Pod	Dec. 28-29	4	4	12	57	27	0	100	
	Dec. 31-Jan. 1	6	0	0	0	2	98	100	
Caseknife	Dec. 28-29	4	6	20	46	28	0	100	
	Dec. 31-Jan. 1	6	0	0	0	2	29	31	
Bountiful	Dec. 28-29	4	6	14	76	4	0	100	
	Dec. 31-Jan. 1	6	0	2	5	1	92	100	
Plentiful	Dec. 28-29	4	4	20	76	0	0	100	
	Dec. 31-Jan. 1	6	0	4	5	7	84	100	
Pinto (650)	Dec. 28-29	4	4	37	55	4	0	100	
	Dec. 31-Jan. 1	6	0	0	0	1	56	57	
Davis' White Wax	Dec. 28-29	4	3	43	45	9	0	100	
	Dec. 31-Jan. 1	6	0	0	0	8	92	100	
Dwarf Horticultural	Dec. 28-29	4	13	45	24	0	0	82	
Alabama No. 1	Dec. 28-29	4	2	12	0	0	0	14	
<i>Nicotiana tabacum</i> var. Gold Dollar	Dec. 28-29	4	6	28	59	7	0	100	
	Dec. 31-Jan. 1	6	0	2	1	3	94	100	

and 8 consistently fewer parasites entered the plants than with 1A. This difference was even greater than the table indicates because totals of less than 100 represent all the parasites harbored by a plant while totals of 100 do not. In previous experiments with *T. kok-saghyz* grown from seed,

TABLE 5.—Comparative development of the root-knot nematode (populations 1A, 2, 5, 8) in *Taraxacum kok-saghyz* Rod.

Pop. No.	Period of development		Number of parasites in each group					
	Date infected	Time	A	B	C	D	E	Total
	1942-43	weeks						
1A	Nov. 9-10	4	4	19	18	6	0	47
	do	6	9	14	25	34	18	100
	Jan. 18-19	6	11	16	29	20	24	100
	do	6	15	30	21	14	20	100
	do	6	2	13	16	18	51	100
	do	6	5	37	54	2	2	100
	Nov. 9-10	8	4	16	28	1	51	100
	do	4	0	1	0	0	0	1
2	do	6	1	3	2	7	0	13
	Jan. 18-19	6	3	9	15	13	16	56
	do	6	1	4	14	24	40	83
	do	6	6	9	5	16	40	76
	Nov. 9-10	8	4	8	13	0	12	37
5	Jan. 22-23	6	0	6	0	0	44	50
	Nov. 9-10	4	9	6	4	0	0	19
	do	6	0	18	14	12	8	52
	do	8	0	0	1	0	7	8

results suggested the possibility that individual plants differed in their relations to the parasite. In view of these previous results it is interesting to note the conspicuous developmental lag that occurred in one of the plants infected with population 1A (Table 5).

Larvae of population 1A entered the roots of cotton freely but development was sharply retarded and was not much more advanced at the end of 8 weeks than at the end of 4 weeks (Table 6). The development of population 2 was moderately rapid. The development of population 1A in alfalfa (Table 7) was about the same as the development of population 2 in cotton

TABLE 6.—*Comparative development of the root-knot nematode (populations 1A and 2) in cotton (variety Coker 100)*

Pop. No.	Period of development		Number of parasites in each group					
	Date infected	Time	A	B	C	D	E	Total
	<i>1942-43</i>							
		<i>weeks</i>						
1A	Nov. 16-17	4	48	52	0	0	0	100
	Nov. 19-20	4	85	15	0	0	0	100
	Jan. 15-16	4	63	10	0	0	0	73
	do	4	83	17	0	0	0	100
	Nov. 16-17	6	46	52	2	0	0	100
	Nov. 19-20	6	18	21	3	0	0	42
	Nov. 23-24	6	59	41	0	0	0	100
	Jan. 15-16	6	75	25	0	0	0	100
	Jan. 15-16	8	56	44	0	0	0	100
	Jan. 20-21	8	32	67	1	0	0	100
	do	8	22	77	1	0	0	100
	do	8	22	77	1	0	0	100
2	Nov. 16-17	4	5	30	34	31	0	100
	Nov. 19-20	4	15	71	13	1	0	100
	Jan. 15-16	4	13	64	23	0	0	100
	do	4	16	58	16	10	0	100
	Nov. 16-17	6	4	25	31	28	12	100
	Nov. 19-20	6	0	18	33	30	19	100
	Nov. 23-24	6	2	15	26	34	6	83
	Jan. 15-16	6	5	23	32	31	9	100
	do	8	0	3	10	20	67	100
	Jan. 20-21	8	0	5	11	14	70	100
	do	8	0	2	13	22	63	100
	do	8	0	3	21	26	50	100

but 2 failed to infect alfalfa to any appreciable extent. This failure is not surprising since larvae of other populations have failed to enter the roots of certain plants, or have entered in very small numbers, but the development of the few parasites of population 2 found in alfalfa does not appear to have been appreciably retarded. Differences in the number of parasites harbored by the different plants in this and other experiments were due, in some measure, to differences in the number of larvae to which they were exposed. Furthermore, the root system of alfalfa seedlings is less branched and has fewer root tips than that of many plants, and the roots grow downward and coil in the bottom of the pot. It was difficult to infect alfalfa with an adequate number of parasites regardless of the population employed. However, neither the character of the root system nor the number of larvae to which it was exposed can account for the almost complete failure to infect

alfalfa with population 2. That this population included more than one race is, admittedly, a possibility.

SUPPLEMENTARY OBSERVATIONS AND DISCUSSION

Many plants are regarded as "resistant," not because larvae fail to enter but because they fail to develop after entering. Barrons (1) found no significant difference between the mean number of larvae per rootlet in such "resistant" plants as corn (*Zea mays* L.), oats, rye, onion, *Crotalaria* (*Crotalaria spectabilis*), and bean (var. Alabama No. 1) and such "susceptible" plants as cowpea (var. Cream Crowder), okra, bean (var. Kentucky

TABLE 7.—Comparative development of the root-knot nematode (populations 1A and 2) in alfalfa

Pop. No.	Period of development		Number of parasites in each group					
	Date infected	Time	A	B	C	D	E	Total
	1942-43	weeks						
1A	Oct. 26-27	4	4	32	13	0	0	49
	Oct. 29-30	6	1	25	29	42	3	100
	Jan. 4-5	6	0	2	3	19	9	33
	Jan. 25-26	6	0	3	8	7	22	40
	do	6	1	7	19	13	38	78
	do	6	0	3	6	25	66	100
	Nov. 2-3	8	0	4	15	16	65	100
	Jan. 1-2	8	0	1	7	5	26	39
	do	8	0	0	3	27	70	100
	Jan. 4-5	8	0	0	1	0	56	57
	do	8	0	0	2	7	81	90
2	Oct. 29-30	6	0	0	0	0	0	0
	Jan. 4-5	6	0	0	0	0	0	0
	Jan. 25-26	6	0	1	1	5	2	9
	do	6	0	0	1	1	0	2
	do	6	0	0	1	1	0	2
	Nov. 2-3	8	0	0	0	0	0	0
	Jan. 2-3	8	0	0	0	0	1	1
	do	8	0	0	0	0	0	0
	Jan. 4-5	8	0	1	0	0	0	1
	do	8	0	0	0	0	0	0

Wonder), and tomato, when all were equally exposed to infection. Results from the present experiments indicate, nevertheless, that larvae do not enter the roots of some plants as readily or in as large numbers as they do others and the existence of plants immune to invasion, at least by some races, seems a likely possibility.

The inoculum potential of the soil may be influenced, no doubt, by many factors but it is directly dependent, initially, on the egg production of the parasites. Conspicuous galling on the roots of a crop may not be an infallible indication that a following crop of the same kind will be equally affected. Subsequent generations of the parasite develop from eggs, not from galls. A plant may have galling of considerable severity yet development of the parasites may be sufficiently retarded to result in a pronounced reduction or, in some cases, an almost complete suppression of egg-laying

and this will be reflected, eventually, by a corresponding decline in soil infestation. It is erroneous to assume that such a decline cannot occur if the crop is one of those that have come to be regarded as highly "susceptible." No plant is an equally suitable host for all races.

The influence of some of these effects of the host on the parasite became manifest, unexpectedly, when attempts were made to build up and maintain different populations for experimental purposes. When tomato (*Lycopersicon esculentum* Mill.) was used as a maintenance plant the initial suc-

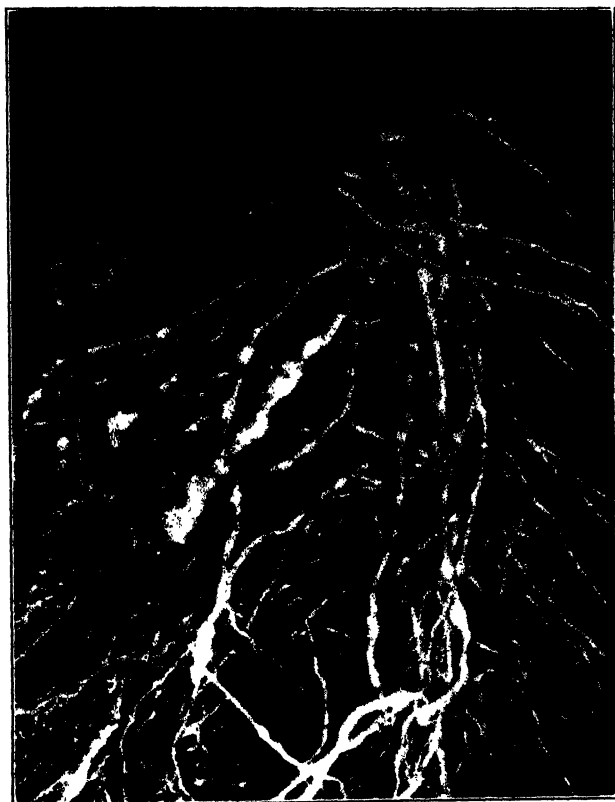


FIG. 2. Root system of a plant of *Nicotiana glauca* that had been growing for 61 days in soil infested with the root knot nematode, population 8.

cess, with a few exceptions, seemed fairly satisfactory but eventually it became evident that all populations were not being maintained at a high level. For example, when potato tubers infected with population 3D were used as inoculum, a very severe infection could be produced on tomato. When the roots of a tomato plant thus infected were used as inoculum to infect another tomato plant the resulting infection was less severe. With repeated transfers from tomato to tomato the severity of the infection declined until it became moderate to light. Somewhat similar results were obtained when attempts were made to segregate and build up populations from single egg masses, of which the following is an interesting example.

Eight small plants of common tobacco were inoculated by placing among the roots of each a single egg mass of population 3B, and 8 similar plants were inoculated in the same manner with population 3C. These two populations were known to produce severe galling on tobacco and previous work had given no intimation that tobacco was not equally suitable for, and equally affected by, both. All the plants came from the same lot



FIG. 3. Root systems of tobacco plants infected with the root-knot nematode. When these plants were small a single nematode egg mass was placed among the roots of each, the upper row being infected in this manner with population 3B and the lower row with population 3C. Photographed 15 weeks later.

of seedlings, were approximately the same size, were inoculated at the same time, were potted in the same sized pots using the same kind of soil, and were grown on the same greenhouse bench. All plants within each group grew at about the same rate, maintaining approximately the same size but, by the end of 4 or 5 weeks there was a very noticeable difference between the groups, those inoculated with population 3B being larger. This dif-

ference was maintained throughout the growth of the plants, eventually becoming very conspicuous. After 15 weeks all plants were removed from the pots and the roots washed free of soil. The degree of galling within each group was remarkably uniform but the plants harboring population 3C were much more heavily infected and much more severely galled than those harboring population 3B. The photographs reproduced in figure 3 show fairly well the pronounced difference between the groups with respect to galling but they do not show the even more pronounced difference with respect to egg production. Egg masses were present on the roots of the plants infected with population 3B but there were comparatively few, while on the plants infected with population 3C they were exceedingly abundant. When these plants were inoculated the egg masses used had been selected as being large and of uniform size but the number of eggs per egg mass was not determined. It was conceivable, therefore, that the egg masses selected from population 3C contained a larger number of eggs than did those selected from 3B. To secure some indication regarding the likelihood that this was the case, counts were made subsequently on egg masses similar to those used to inoculate the plants. In round numbers, eggs per egg mass varied from about 300 to 500 with no apparent difference between populations.

CONCLUSIONS

Some plants are highly suitable hosts for a given race of the root-knot nematode and others are highly unsuitable hosts, but most lie between these two extremes and nearly every degree of suitability may be encountered. Retarded development of the parasite is a manifestation of unsuitability or (if we define resistance in a plant as its condition of being an unsuitable host) of resistance. There appears to be no correlation between the suitability of the host and the freedom with which larvae enter its roots. Many plants that are highly unsuitable are invaded as freely as more suitable ones. The present experiments indicate that, even though given an ample opportunity, larvae enter some plants in very small numbers although it remains to be demonstrated that any one of the flowering plants is immune from invasion. Results with alfalfa raise some doubts that failure of larvae to enter a plant in large numbers can be accepted as *prima facie* evidence of unsuitability. There appears to be a direct correlation between suitability of the host and rate of parasite development and a direct correlation between rate of parasite development and egg production. Where parasite development is only slightly retarded the effect may be little more than to reduce the number of generations that occur in a given period. When development is more strongly retarded many of the females may never reach maturity and there may be a reduction in the egg output of those that do. In extreme cases development is almost completely suppressed and, of course, eggs are not produced. Unsuitability of the host

with its various consequences is not necessarily accompanied by a corresponding reduction in severity of galling.

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TEMPERATURE EFFECTS ON THE EXPRESSION OF THE YELLOW S VIRUS IN SOUR CHERRIES

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The virus yellows of sour cherries has long been present in New York State orchards. Stewart (9) accurately described the symptoms. He stated that the trouble occurred occasionally in sour cherries and ascribed it to "unfavorable soil and weather conditions." The disease has been reported in *The Weekly News Letter*¹ as occurring in varying amounts since 1921. Crosby, Mills, and Blauvelt (1) described the disease as a physiological leaf drop "apparently correlated with winter injury" in their bulletin and in subsequent annual revisions until Keitt and Clayton (6, 7) demonstrated that a bud-transmissible virus was responsible, after which it was referred to (2) as virus leaf drop. Hildebrand and Mills (5) reported that the yellowing and dropping of leaves caused by the virus yellows was prevalent in New York during 1928, 1931, 1935, 1937, and 1938. This report was based on the plant disease records of the writer for the years 1928 to 1940.

Subsequent studies have been made of *The Weekly News Letter* and the fruit disease questionnaires, which have been filled out annually in the commercial fruit-growing counties since 1931. These records indicate that in 8 of the last 25 years the symptoms of yellows have been unusually severe. These years were 1921, 1927, 1928, 1931, 1938, 1941, 1942, and 1945. Distinctly less yellows occurred in 1935 and 1937 and these two years were placed in the moderate group after careful study of the available records.

Hildebrand (4) stated that virus yellows symptoms were obscured by defoliation from fungus leaf spot in 1943. It seems evident that some other factor than the incidence of cherry leaf spot (*Coccomyces hiemalis*) must be sought to explain the variation in the amount of the yellows symptoms during the last 25 years in this State. During the 8 separate years when yellows symptoms were unusually severe, leaf spot was severe in 3 years, slight to moderate in 2 years, and very slight to a trace in 3 years. In the 16 years when the yellows symptoms were less intense, the leaf spot was severe in 4 years, slight to moderate in 7, and very slight to a trace in 5 years.

A possible explanation for the wide variation in the expression of the yellows virus from year to year in the orchard appears in the greenhouse experiments of Keitt and Moore (8) who found that there is a masking of the yellows symptoms when trees which have been budded with infected buds are held at high temperatures. Yellows symptoms were freely expressed in the budded trees held at 16° C. (60.8° F.), only one budded tree showed the symptoms when held at 20° C. (68° F.), and no trees budded

¹ *The Weekly News Letter* is a mimeographed compilation of weekly reports on disease and insect development in New York State, issued since 1919.

and held at 24° C. or 28° C. developed the yellows symptoms. These data indicate that partial or complete masking of the symptoms occurs when the temperature rises much above 61° F.

The records mentioned above were tabulated for Montmorency cherry orchards near Lake Ontario to show, for each of the last 25 years, the date of the beginning of bloom, of the petal-fall stage, and of the first appearance of the yellows symptoms. From the climatological data of the Rochester office of the United States Weather Bureau, the mean temperatures were determined for 10-day periods in relation to the petal-fall stage for each year. Figures for the 8 years when the yellows symptoms were most severe were placed in one group, and figures for the 17 remaining years were in a second group. The two groups were then treated statistically to determine whether any significant difference existed for any of the 10-day periods between the years when yellows symptoms were strongest and the years when the symptoms were less pronounced. In table 1 the mean temperatures for the various periods are given for the 8 years of conspicuous yellows symptoms and for the 17 years of lighter symptoms. The F values and the odds are included to show the relative significance of the differences in mean temperatures. It will be noted (Table 1) that the mean temperature for each period was always lower for the years when heavy yellows symptoms occurred than for the years of lighter yellows expression. The first, second, and third 10-day periods following the petal-fall stage show a very significantly lower temperature for the years of severe yellows. The 10-day period preceding the petal-fall stage and the period 31 to 40 days after the petal-fall show only a probable significant decrease in mean temperature for the years of severe yellows. Various 20- and 30-day periods in relation to the petal fall are included in table 1. All but one of these periods show a very significantly lower mean temperature for the years when the yellows was most severe. As might be expected from the differences shown by the 10-day periods, the 30-day period following the petal fall shows the most significant differences in temperature as indicated by the highest F value of 33.01.

Before the temperature records were compiled, it was noted that the years of earliest cherry bloom were also the years in which the most severe yellows appeared. The average number of days after April 10 for the beginning of bloom was 18.12 for the 8 years of heavy yellows and 32.41 for the 17 years of lighter yellows with a highly significant F value for the difference of 20.85. In other words, the average calendar date for the beginning of bloom in the 8 years of heavy yellows was April 28, which was very significantly earlier than May 12, the average date for the years of lighter yellows symptoms. The average date of the petal-fall stage was May 12 for the years of heavy yellows, very significantly earlier than the average date of May 25 for the years of lighter symptoms, as shown by an F value of 17.51. Sour cherries began to bloom in the Lake Ontario cherry belt on April 16 in 1945 and the writer ventured to predict at that time that,

with one of the earliest bloom periods on record, we should expect heavier yellows in 1945 than in the two previous years, since in 1943 the bloom was later than average and in 1944 only a few days earlier than average. This 1945 season proved to be the year with the most severe yellows on record. The reason for this is obvious. Leaf development in sour cherries starts at about the petal-fall stage and is rapid during the next few weeks. An early bloom period, as in 1945, is followed by this leaf development in May when the normal monthly mean temperature is about 56° F. instead of occurring largely in June when the normal monthly mean is about 66° F. in this area.

The relation between the date of bloom and the average temperature for the 30 days after petal fall was measured by computing the correlation

TABLE 1.—*Mean temperatures of various periods in relation to the petal-fall stage in 8 years of heavy yellows symptoms and in 17 years of lighter symptoms for the 25 years of 1921 to 1945*

Ten day period	Mean temperature, degrees F.		F	Odds
	8 years, heavy yellows	17 years, light yellows		
0- 9 days before petal fall	51.99	59.29	5.74	19 to 1
1-10 days after petal fall	55.35	62.85	11.01	99 to 1
11-20 do	58.89	65.62	14.32	99 to 1
21-30 do	63.84	68.62	13.78	99 to 1
31-40 do	64.65	69.49	5.98	19 to 1
10 before to 10 after	55.19	61.08	7.74	19 to 1
1-20 days after petal fall	57.12	64.25	20.78	99 to 1
1-30 do	59.32	65.68	33.01	99 to 1

between the number of days after April 10 when the bloom began with the mean temperature of this 30-day period after the petal-fall stage. For the 25 years the coefficient of correlation was .0789, giving odds of 99 to 1 that the temperatures are correlated with the bloom date. The regression was + 0.31, so that for each day later the bloom began the average temperature of the 30-day period rose three-tenths of a degree Fahrenheit. The correlation of the date of the petal-fall stage and average temperature for the following 30 days also was computed. The correlation coefficient was highly significant ($r = 0.732$) and the regression value of + 0.30 again showed a rise of three-tenths of a degree for the 30 days after petal fall for each day the petal-fall stage was delayed.

While high temperatures in the 30 days following the petal-fall stage decrease the yellows symptoms, higher than normal temperatures in March and April tend to increase the yellows symptoms which appear later in the orchard. This apparent contradiction is due to the fact that high temperatures early in the season cause an earlier bloom and this earlier bloom is followed in most years by lower temperatures after the petal-fall stage and

a consequent greater manifestation of yellows symptoms. This is shown by a highly significant correlation of the mean temperature for March and April with the date of the petal-fall stage. The correlation coefficient for the 25 years is -0.7684 and the regression value -2.01 days. For each increase of 1 degree in the mean temperature the petal fall occurs 2 days earlier. The monthly mean temperatures for March, April, May, and June for the years of heavy and light yellows are in table 2. The increase in the mean temperature for March in the years of heavy yellows is not quite enough for odds of 19 to 1. The F value is 4.26 when 4.28 is required for these odds. The increase in the mean temperature for April in the years of heavy yellows is highly significant as shown by the F value of 15.74, well over the value required for odds of 99 to 1. No significant differences occur in the mean temperatures for May and June between the years of heavy yellows and those of light symptoms.

TABLE 2.—Mean monthly temperatures for the 8 years of heavy yellows and the 17 years of lighter symptoms

Month	Mean temperature, degrees F.		F	Odds ^a
	8 years, heavy yellows	17 years, light yellows		
March	36.7	33.6	4.26	n.s.
April	48.2	43.5	15.74	99 to 1
May	57.2	56.9		n.s.
June	66.4	67.3		n.s.

^a n.s. indicates no significance.

The possible additional effects of the prevailing temperatures during the time the yellows symptoms appear, on the severity of the expression of symptoms was investigated. The mean temperatures of 10-day periods in relation to the date of first appearance of the yellows were computed for the 8 years of most severe yellows and for 14 years when the yellows symptoms were less prominent. In three years of light yellows the date of first appearance was not recorded. These mean temperatures, in relation to the time of appearance of yellows symptoms, are given in table 3.

The only period in which the temperatures show a probable significant difference is the 10-day period just preceding the appearance of yellows. This period is approximately the same as the 21–30-day period after petal fall (Table 1).

The 10-, 20-, or 30-day periods after yellows symptoms first appear show no significant differences in temperatures for years of heavy and light yellows. These data indicate that the prevailing temperatures during the time of appearance of yellows symptoms do not appreciably affect the magnitude of these symptoms.

TABLE 3.—*Mean temperatures of various periods in relation to the first appearance of yellows symptoms for 8 years of severe and for 14 years of light yellows*

Ten-day period	Mean temperature, degrees F.		F	Odds ^a
	8 years, heavy yellows	14 years, light yellows		
0- 9 days before 1st yellows	63.90	69.38	7.2	19 to 1
1-10 days after 1st yellows	68.42	70.46	2.8	n.s.
11-20 do	72.81	72.20	< 1.0	do
21-30 do	72.62	72.70	< 1.0	do
1-20 do	70.64	71.31	< 1.0	do
1-30 do	71.31	71.78	< 1.0	do

^a n.s. indicates no significance.

The possibility that the amount of precipitation might influence the appearance of yellows symptoms also was investigated. The total precipitation for January, February, March, and April did not differ significantly for the years of heavy and of light yellows as shown by an F value of only 1.2. The total precipitation was computed for all the periods used in the temperature studies in relation to the petal-fall stage (Table 1), and to the time of appearance of symptoms (Table 3). No consistent increases or decreases in precipitation occurred in the years of heavy yellows as compared with the years of light or moderate yellows, and in no period did the difference in precipitation approach even probable significance. It appears that no measurable effect on the expression of yellows in the 25 years was the result of variations in precipitation.

CONCLUSIONS

The chief factor determining the expression of virus yellows in the orchard in any given season is the prevailing temperature during the 30-day period following the petal-fall stage. The effect of early bloom in increasing the expression of yellows symptoms is due to the lower temperatures normally following such an early bloom. The prevailing temperatures after yellows symptoms first appear do not affect the degree of these symptoms. The amount of precipitation in any season does not appear to affect the severity of yellows symptoms. The incidence of cherry leaf spot also does not appear to influence the appearance of yellows symptoms.

Since prevailing temperatures following the petal-fall stage cause a great variation in the apparent amount of virus yellows present in an orchard from year to year, it appears that no data observed to date, based on the apparent incidence of yellows in two different years, prove or disprove spread of the cherry-yellows virus after the trees are set in the orchard.

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MOSAICS OF WINTER OATS INDUCED BY SOIL-BORNE VIRUSES

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INTRODUCTION

In 1943, the writer received diseased winter-grown Quincy Red oat plants that had been sent to the Department by J. L. Seal, Pathologist at the Alabama Agricultural Experiment Station. These plants were approaching maturity and were dead when received, but the leaf symptoms were suggestive of mosaic. Another lot of diseased plants was submitted by Dr. Seal and inoculations were made into oats, wheat, and corn. The results were negative. In the late spring of 1944, Seal submitted diseased plants again. These were transplanted, but they were not thrifty. The symptoms were unmistakably typical mosaic. However, attempts to effect a transmission failed. In the spring of 1944 R. E. Atkinson of the Emergency Plant Disease Survey, of the Bureau of Plant Industry, Soils, and Agricultural Engineering, sent winter oat plants to Beltsville, Md., from South Carolina and later the writer collected mosaic plants from points in North Carolina and South Carolina. All of these collections were potted and cultured in a greenhouse. Although the symptoms were characteristic of mosaic, all attempts to transmit a virus again met with failure.

On a basis of these failures, it was concluded that the viruses of Brome mosaic (*Marmor graminis* McK.)² and of the wheat streak mosaics (*M. virgatum* McK.)² which infect oats, corn, and wheat at summer temperatures, probably were not the causes. Adequate facilities for obtaining low-temperature culture conditions were not available for testing inoculations at temperatures near 60° and 65° F., the optimum range for the soil-borne viruses infecting wheat.

DISTRIBUTION, RECURRENCE, AND IMPORTANCE

Oat mosaic has appeared in four successive seasons on the same land in a limited area at Auburn, Ala., and in two successive years on the same land at Hartsville and Clemson, S. C., and Statesville, N. C.

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The author is indebted to Matthew Koerner for assisting in conducting the tests; to J. L. Seal and T. H. Rogers of the Alabama Agricultural Experiment Station; R. P. Bledsoe of the Georgia Agricultural Experiment Station; G. H. Armstrong and W. R. Paden of the South Carolina Agricultural Experiment Station; G. K. Middleton of the North Carolina Agricultural Experiment Station; J. W. Hendricks of the North Carolina Department of Agriculture and to Coker's Pedigreed Seed Co., Hartsville, South Carolina, for supplying seed, infested soil, plant materials, and other facilities. Most of the seed used in the tests was provided by T. R. Stanton, Senior Agronomist in charge of oat investigations, of this Division.

² McKinney, H. H. Descriptions and revisions of several species of viruses in the genera Marmor, Fraetilinea, and Galla. Jour. Washington Acad. Sci. 34: 322-329. 1944.

During the season of 1944, R. E. Atkinson made a survey in many fields of oats in the Carolinas, and in his report,³ he indicates the presence of oat mosaic in 9 counties in North Carolina and in 10 counties in South Carolina.

During the season of 1945, the writer examined many oat fields in South Carolina, and found mosaic in Pickens and Darlington Counties. During the same season, A. E. Prince of the Emergency Plant Disease Survey made a survey of oat fields in the Carolinas and found oat mosaic in Oconee, Pickens, and Darlington Counties in South Carolina and in Iredell County, North Carolina.

The disease was more severe in 1944 than in 1945. Susceptible varieties such as Letoria (C.I. 3392), Bond (C.I. 2733), Camellia (C.I. 4079), and Victoria (C.I. 2401) were greatly retarded and severely damaged in 1944, whereas in 1945 these varieties showed much less damage, even though infected.

As in the wheat mosaics, seasonal conditions, especially temperature, appear to influence the amount of damage caused by oat mosaic. Under suitable conditions the disease is capable of causing severe damage to certain varieties. It appears that control can be effected through resistant lines and varieties.

SPREAD OF OAT MOSAIC

By soil

Soils in which mosaic oats had grown were collected at Auburn, Ala., and Statesville, N. C., transported to Beltsville, Md., and placed in separate wooden bins, located out of doors. The bins were 48 inches long, 12 inches wide, and 12 inches deep. Similar bins placed nearby were filled with non-mosaic soil from Beltsville to serve as controls.

On October 6, 1944, seed of Letoria (C.I. 3392), Iowa Winter Sel. 8 (C.I. 4467), and Victoria Rainbow (C.I. 4191) oats were sown in the bins.

On November 25, 40 days after seeding, samples of plants were removed from the bins. These seedlings and roots were thoroughly washed and disinfected in a solution of 1 part mercuric chloride in 999 parts of water. After this treatment, the seedlings were transplanted to steamed, fertile potting soil and then grown in a greenhouse controlled for 65° F.

On December 1, four of the plants removed from the soils obtained at Auburn, Ala., and Statesville, N. C., showed mosaic. No mosaic appeared in plants removed from the Beltsville soil (Fig. 1).

On December 9, the bins of infested and noninfested soils with the remaining plants, were brought into the greenhouse (65° F.). A few of the plants had a faint mottling at this time, and within 10 days mosaic was very evident in all of the plants in the Auburn and Statesville soils, whereas all plants growing in Beltsville soil remained perfectly healthy. Subsequent tests indicate that 60° is more favorable than 65° F. for inducing mosaic from the soil, but 65° has been more favorable for the full expression

³ Atkinson, R. E. A new mosaic chlorosis of oats in the Carolinas. U. S. Dept. Agr., Plant Dis. Repr. 29: 86-89. 1945.

of symptoms. The results of these tests demonstrate that the oat-mosaic-inducing agent is associated in some way with the soil, and that the agent overseasons in the soil.

Wheats known to be highly susceptible to the soil-borne wheat mosaic viruses, *Marmor tritici* H. emend McK.,⁴ failed to develop mosaic when sown



FIG. 1. Oat plants of the hybrid Victoria \times Rainbow. All plants grown concurrently out of doors for 40 days during the autumn, and in a greenhouse during the winter at Beltsville, Md. Photographed 76 days after seeding, 36 days after transplanting. Left: healthy plants cultured in field soil that does not induce mosaic. Right: diseased plants cultured in field soil in which mosaic oat plants had grown the previous crop season, then transplanted to steamed greenhouse soil. These plants had mosaic mottling.

At time of transplanting, the underground parts of both lots of plants were washed thoroughly, placed in a 1:1000 solution of mercuric chloride in water for 10 minutes, and transplanted without rinsing in water.

in soil carrying the oat-mosaic agents at Statesville, N. C., Clemson, S. C., Hartsville, S. C., and Auburn, Ala., while adjacent rows of oats developed high percentages of mosaic. Varieties of winter oats now known to be susceptible to the oat mosaics under discussion have failed to develop mosaic when sown in soil carrying wheat-mosaic viruses at Arlington Experimental Farm, Va., and near Marshall, Ill. Winter oats have been tested in soils

⁴ See footnote 2.

infested with wheat-mosaic viruses at several other points farther north in Illinois and at Lafayette, Indiana, but winter survival was always low or nil. Mosaic has never been observed in any surviving oat plants in these tests.⁵



FIG. 2. Letoria oat plants illustrating nontransmission of mosaic through seed from mosaic plants. Left: typical plant from seed of mosaic plants cultured in non-mosaic-inducing soil. Center: typical plant from seed from mosaic-free plants. Right: typical diseased plant, stunted by mosaic, from seed of mosaic-free plants cultured in field soil in which mosaic oat plants had grown the previous crop season.

Tests with Seed from Diseased Plants

Seed of the varieties, Letoria (C.I. 3392), Victoria \times Rainbow (C.I. 4191), and Iowa Winter Sel. 8 (C.I. 4467) was collected from field-grown plants

⁵ Cooperative tests with Benj. Kochler and Ralph M. Caldwell of the Illinois and the Purdue Agricultural Experiment Stations, respectively.

having severe mosaic symptoms. In the autumn of 1944 these lots were sown outdoors in bins of nonmosaic soil at Beltsville, Md. Each lot of seed was sown at the rate of 1 gram per foot in 1 row 48 inches long. The stands were excellent and remained so during the test. On December 9 these bins were removed to a greenhouse controlled near 65° F. The plants were thrifty and remained completely free of mosaic throughout their life (Fig. 2). Throughout the test, these plants were near the mosaic plants growing in the infested soils.

INOCULATION TESTS

In August, 1944, soil from an oat-mosaic infested plot at Auburn, Ala., was planted to Letoria oats at Beltsville, Maryland. Culture was in 6-inch earthen greenhouse pots in a chamber controlled near 60° F. On October 19, the pots and plants were transferred to a greenhouse in which the temperature was 65° F. during the night and overcast days. On November 6, mosaic was evident in most of the plants. On November 14, 74 healthy young seedlings of Letoria oats in 2-3-leaf stage were inoculated with an extract made from finely pulped leaf and sheath tissue from the mosaic plants. The tissue was pulped with quartz sand in a mortar, with sufficient water to make inoculum the consistency of a thick porridge. Carborundum powder (600-grain) was added to the inoculum. Thumb and forefinger were dipped in this mixture and used to wipe the leaves of the seedlings, and the residue was rinsed off with water.

After inoculation the plants were cultured at 60-65° F. The first signs of mosaic appeared 13 days after inoculation. Of the 74 seedlings inoculated, 17, or nearly 23 per cent, developed mosaic.

A total of 3,273 oat plants have been inoculated with extracts from mosaic oat plants, and 972 plants (29.7 per cent) became infected. Single sources have been subtransferred successfully to oats as many as 3 times.

The early failures to transmit oat mosaics are attributed largely to the fact that suitable temperatures were not available for the culture of the seedlings before and after inoculation. When cultured at temperatures much above 60° F., the leaves of oat seedlings are very tender and subject to severe injury from the inoculation technic. Such injuries tend to interfere with increase and movement of the virus.

Of 40 inoculation tests made since the first successful inoculation, 3 tests have failed completely, and only one has given 100 per cent infection, with 11 plants in that test. Thus far, these oat-mosaic viruses have been much more difficult to transmit manually than the soil-borne wheat-mosaic viruses.

GENERAL SYMPTOMS IN PLANTS INFECTED FROM SOIL

The first signs of oat mosaic in the field appear as soon as the plants start growth in the spring. In 1945, at Auburn, Ala., the first signs were evident the second week in January, and at Statesville, N. C., they were just appearing the first week in March.

The leaves manifest chlorotic streaks, spots, and patches. Depending on the variety of oats and the seasonal conditions, the chlorosis ranges from a very faint light green to yellow or ashen gray. The patterns and their persistence vary somewhat with the oat variety, the seasonal conditions, and the predominating type of virus. In 1944, the chlorosis was generally more severe and more persistent; also there was more stunting of plants than was the case in 1945.

In Letoria and Victoria \times Rainbow, the two varieties studied thus far, the symptoms were most severe at 60 to 65° F., 65° being slightly more favorable for chlorosis than 60°. As the temperatures reached 70° F. and above, the new leaves had weak symptoms or none at all. However, assays on such plants revealed virus in flag leaves that had no visible signs of mosaic.

Mosaic plants growing in pots in the greenhouse and out of doors during the spring showed marked fluctuations in the expression of mosaic in growing leaves with changes in temperature. During cool periods the symptoms were much more evident than during warm periods.

In the field, several varieties of oats such as Appler (C.I. 1815) manifest extremely mild, light-green mottling and sometimes none. A few hybrids, Palestine \times Dawn (C.I. 4230) and two sister hybrids Lee \times (Bond-Loggold) C.I. 4212 and C.I. 4214, developed rosette symptoms in portions of the populations.

SYMPTOMS INDUCED BY TWO ISOLATES FROM DISEASED PLANTS

Owing to the difficulty of effecting manual transmission, symptom reactions are the only properties of the virus isolates that have been studied.

Apical-mosaic Virus

The isolate here described originated from an oat plant growing in virus-infested soil collected on the Piedmont Branch Experiment Station, Statesville, North Carolina.

In Letoria and Victoria \times Rainbow oats, the symptoms range from light green to yellow narrow lines to typical grass-mosaic type of mottling and blotching. The chlorotic patterns tend to be confined to the top 1 to 3 leaves of the plants, and the patterns tend to be massed towards the distal portion of the leaf as illustrated in figure 3. The emerging leaf commonly has the strongest chlorosis, and usually this is in the form of short broken lines. At 65° F., the severity of the chlorosis gradually reduces with the development of the leaf, and may disappear completely, tending to disappear last from the distal region of the leaf. However, sometimes tips of these leaves have become completely chlorotic prematurely when the plants were transferred to 80° F. When high temperatures continued, the new foliage failed to show signs of the disease. Upper leaf sheaths sometimes manifest mottling for a few days at cool temperatures.

Apical mosaic seems to be the predominating oat mosaic in all of the



FIG. 3. Apical mosaic in leaves of *Lectoria* oats, induced by manual inoculation. The two segments at left illustrate one healthy leaf; the third and fourth segments illustrate one mosaic leaf (both leaves removed just above their ligules). The remaining two segments illustrate the distal halves of mosaic leaves; the proximal halves, not shown, had little or no mottling. (Mag. $\times 1$.)

areas that have been under the writer's observation in the Carolinas, Georgia, and Alabama, and some isolates have induced extremely mild chlorosis in comparison with the type virus.

Eyespot-mosaic Virus

In 1944, eyespot symptoms were noted in all of the mosaic oat fields observed by the writer, but they were complicated with the symptoms of the chlorotic lines and mottling of apical mosaic. In 1945, very little of the eyespot reaction was evident, but some was observed in all of the experimental plats.

The isolate here described originated from a plant growing in the virus-infested soil collected at Auburn, Ala. In *Letoria* and *Victoria* × *Rainbow* oats the fusiform spots or eyespots are essentially comparable with the rings associated with many mosaics of dicotyledonous species. The linear arrangement of the main vascular elements in the grasses doubtless accounts for the fusiform or spindle shape of these spots. The borders of the eyespots and other chlorotic areas frequently become ashen gray, due to the loss of the yellow pigments, and the centers of the eyespots tend to remain green until the normal green portions of the leaf become chlorotic. The spots are most pronounced in leaves that have completed or nearly completed their growth; and in the younger leaves the spotting is more evident in the older or distal portion than it is in the proximal portion. Mottling sometimes appears on the leaf sheaths, but this has been less common with this virus than it has been with the apical mosaic virus. Anthocyanins sometimes develop causing red and purple patterns. Senility is hastened in the diseased leaves. Mosaic leaves of *Letoria* are illustrated in figure 4.

The observations made to date suggest that the eyespot virus continues to invade the leaf tissues for a longer period than does the apical-mosaic virus. Also, the eyespot symptoms tend to be persistent in the leaves, whereas the symptoms of the apical mosaic are very transitory and are seldom evident in the older leaves of the two oat varieties studied. On the other hand, as the greenhouse temperatures rose in the spring, it was observed that the eyespot mosaic failed to appear in the new leaves sooner than the apical mosaic.

NOMENCLATURE OF THE VIRUSES

Marmor terrestre sp. nov.

Specific name from Latin *terrestris*, adj., of the earth, referring to the association of the species with soil.

Host reactions: In *Avena sativa* L., and *A. byzantina* C. Koch, induces chlorotic mottling, streaking, or spotting in varying degrees from mild to severe. *Triticum aestivum* L., has failed to express mosaic in the field when sown in soil infested with *Marmor terrestre*, but when inoculated manually with the type variety of the virus, 2 out of 50 inoculated plants of Michigan Amber wheat expressed mosaic. *Zea mays* L., appears to be immune.

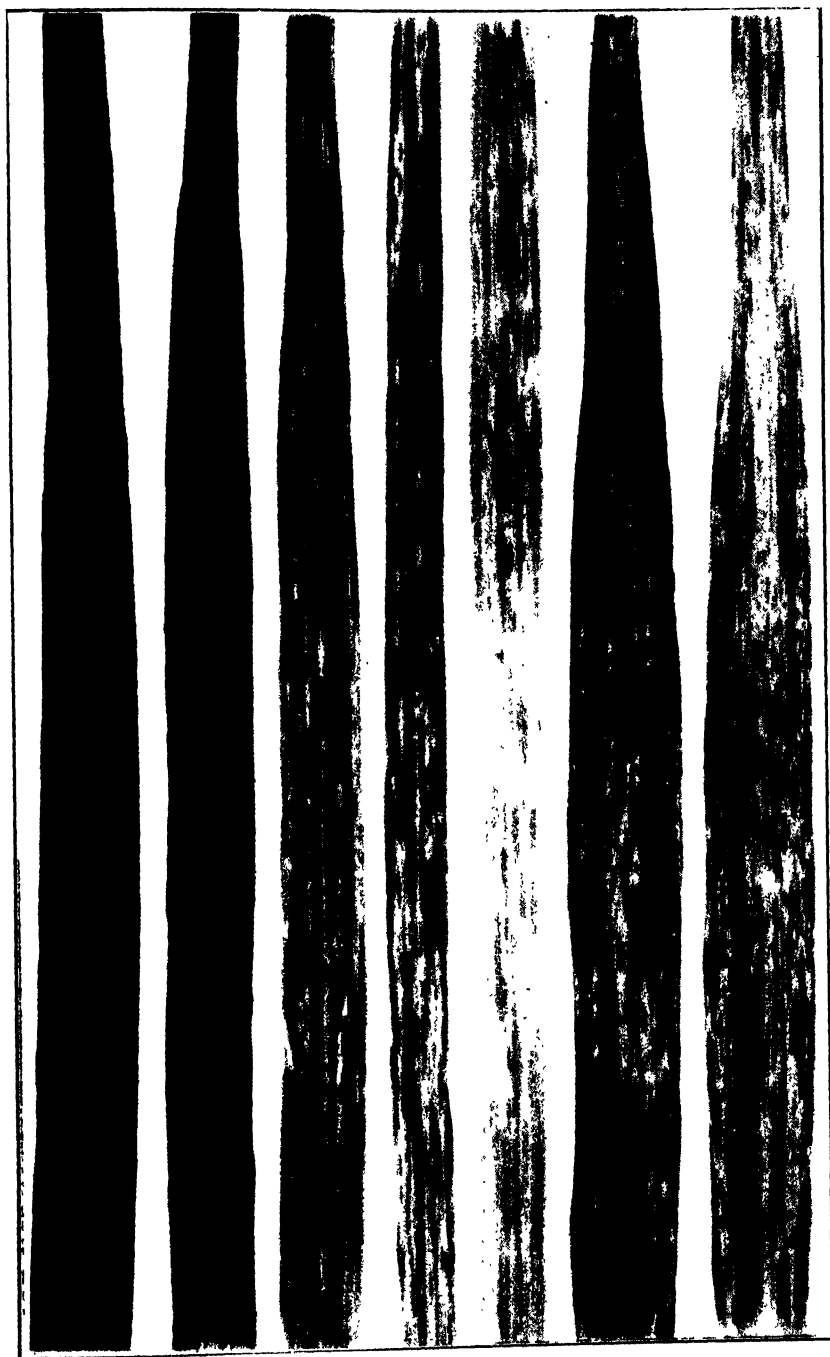


FIG. 4. Eyespot mosaic in segments of leaves of Letoria oats, induced by manual inoculation. Leaf segment from a healthy plant at left. (Mag. $\times 1$.)

Transmission: By inoculation with expressed juice, using the carborundum wiping method, but with difficulty. The incubation period has ranged from 11 to 70 days. This species overseasons in the soil. Infection from the soil has occurred within 35 to 60 days after seeding when plants were cultured out of doors during the autumn and early winter at Beltsville, Md. Mosaic symptoms were expressed when the infected plants were transferred to a greenhouse with the temperature near 65° F. In culture chambers, 60° has been more favorable than 65° F., for infection from the soil, but 65° has been more favorable for the expression of mosaic.

Marmor terrestre, var. **typicum**, var. nov. ♀

Common name: Oat apical-mosaic virus.

Host reactions: In *Avena* spp., agronomic varieties Lectoria and Victoria × Rainbow, cultured at 60°–65° F., the virus induces light-green to yellow dashes and streaks parallelling the long axis of the leaf, and sometimes chlorotic mottling. Chlorotic patterns usually most evident in or near the apical portions of the three upper leaves, tending to fade as the leaves develop. As temperatures reach 75° F., and above, the symptoms become weak and fail to appear in the new foliage. Mosaic leaves are illustrated in figure 3.

Marmor terrestre var. **oculatum**, var. nov.

Common name: Oat eyespot mosaic.

Varietal name from Latin, *oculatus*, adj., eyed, with eye-like marks.

Host reactions: In *Avena* spp., agronomic varieties Lectoria and Victoria × Rainbow, cultured at 60°–65° F., virus induces chlorotic spots and diffuse chlorotic patches. The characteristic eyespots are fusiform or spindle shaped with light-green to ashen-gray borders and green centers. They are most pronounced in leaves that have completed or nearly completed their growth. In the younger leaves the spotting is most evident in the older portions. Mosaic leaves are illustrated in figure 4.

SUMMARY

1. Two mosaics of winter oats occur in limited areas in the Carolinas, Georgia, and Alabama.
2. Manual transmission has been effected by the carborundum-wipe method, but with difficulty.
3. The viruses overseason in the soil.
4. The viruses have not infected wheat in the field, and only one has infected wheat slightly when inoculated manually. None have infected corn.
5. Temperatures near 60° F. for 35 to 60 days seem to be near the optimum for infection through the soil, but 65° F. seems to be near the optimum for the best expression of chlorosis. A temperature of 65° F. has been rather favorable for infection from manual inoculations, and 60° F.

seems to be near the optimum for culturing the seedlings previous to inoculation.

6. The disease caused greater damage to the infected plants in 1944 than in 1945, although the amount of infection seemed to be just as great in 1945. Temperatures were unusually high early in the spring of 1945, which may account for the reduced damage.

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POWDERY MILDEW OF BROAD BEAN CAUSED BY *ERYSIPHE POLYGONI* DC. IN YUNNAN, CHINA¹

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Powdery mildew of broad bean (*Vicia faba* L.) caused by *Erysiphe polygoni* DC. was first collected by the writer on broad bean in Hui-li, Szechwan, in the spring of 1938. Since then it has been found in many places in southwestern China, particularly in Yunnan (7), where the disease is widely distributed and has caused damage for several years in some of the bean-growing sections. This paper presents the results of three years' work on the disease, which, so far as the writer is aware, does not occur, or at least has not been reported yet in the other important bean-growing regions of China.

DISTRIBUTION AND ECONOMIC IMPORTANCE

In Yunnan Province, the disease is of variable importance in different localities. In the northern bean-growing sections, the disease has developed too late in the season to cause appreciable loss; while in the south it is usually present each season and may cause conspicuous blight of stem tips and prematurity of the young pods. In general, the disease is not of great economic importance.

SYMPTOMS

The disease occurs in early spring when the flowering buds have appeared. After a few leaves become covered with mildew, a general infection may occur in the whole plant within a week under favorable conditions.

Small, white, powdery spots appear on the leaves, enlarge rapidly, coalesce to form large areas, and finally cover the entire leaf. Both the upper and lower surfaces of the leaves may become infected. When very young leaves are attacked, they become stunted before attaining one-half their normal size. They usually remain folded longitudinally and at the same time become abnormally thickened. As the disease progresses, these young top leaves become discolored and withered, which results in a blight of stem tips. When old leaves become infected, there is no malformation of the tissues. On infected leaves, both young and old, reddish spots or lesions frequently occur in addition to the characteristic superficial white growth of the fungus.

After the foliage is infected, young stems and petioles become infected. The copious or scant white powdery fungus covers the affected parts and red lesions are common. Old stems, however, are only rarely infected.

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Pods are frequently attacked throughout the growing period of the plant. On old pods, the fungus produces red spots or lesions with or without macroscopic mycelial growth. Young pods, on the other hand, may be severely attacked and they become deformed or ripen prematurely.

In the field, the young leaves are stunted and deformed, affected stem tips are either stunted or killed back, and pods may be lacking or may ripen prematurely.

INOCULATIONS

Potted greenhouse plants were inoculated. They were first atomized with a fine spray of water, then small portions of a young stem bearing a few diseased leaves were laid on, and in contact with, the healthy leaves. Inoculated plants were kept in a moist chamber for 24 to 48 hours. Notes were taken when the development of the disease had reached its maximum stage, usually 7 to 14 days after inoculation.

In March, 1940, four broad bean plants were inoculated with conidia taken from broad beans. Eight days later, all were infected. In another experiment, mildew appeared on 8 of 11 plants 10 days after inoculation. In still another experiment, 24 of 25 plants were infected. Numerous other inoculations were made during subsequent years. Only in rare cases, did the fungus fail to infect the bean plants.

The symptoms on the inoculated plants were somewhat variable at different times of the year. In general, the disease first appeared as grayish white powdery spots which soon covered the entire leaf surface. In certain instances, development of the fungus was rather meager, and it formed only a very thin layer of mycelium just visible to the naked eye. In this case, red lesions were frequent underneath or marginal to the mycelium. Occasionally, only small red spots were seen on the inoculated plants. Young leaves might be killed during the later stages of the disease.

CAUSAL FUNGUS

Powdery mildew of broad bean in Yunnan is caused by *Erysiphe polygoni* DC.

Conidia are borne singly or in short, 2-spore chains on the conidiophores. They are ovate to oblong with round ends, average $38.3\ \mu$ long by $19.1\ \mu$ wide, and vary from 27.9 to $47.0\ \mu$ long and from 12.2 to $22.6\ \mu$ wide. Conidiophores measure 20.4 to $68\ \mu$ by 6.8 to $8.5\ \mu$ and are composed of 2 to 4 cells.

Perithecia are dark brown, globose, and scattered on the mycelium. They are 79.9 to $119\ \mu$ in diameter, with an average of $98.6\ \mu$. Appendages are rudimentary, short and brown, mostly 40 to $48\ \mu$ long and 6 to $8\ \mu$ wide. Asci, varying from 4 to 8, are ovate to broad ovate and are from 30 to $62.6\ \mu$ in length and from 22.6 to $41.8\ \mu$ in width, with an average of 50.9 by $31\ \mu$. Ascospores, 3 to 8, are ovate or longer, broadest in the middle, average 19.5 by $12\ \mu$, and range from 13.9 to 24.4 by 10.4 to $15.7\ \mu$.

Conidia germinate in a wide range of temperature, from 16° to 28° C., within 48 hours. However, they germinate best between 20° and 24° C.

A high relative humidity is essential for the germination of conidia. Fresh spores, placed on dry glass slides in containers with different percentages of relative humidity and incubated at favorable temperatures, germinate only when the humidity is maintained at 90 per cent or above. Very few spores germinate when they are in drops of water. Conidia are generally short-lived. Under the conditions of the present investigation, they were viable only 2 or 3 days in the laboratory.

RELATION OF WEATHER TO DISEASE DEVELOPMENT

The development of powdery mildew may be correlated with weather conditions in Yunnan, because the disease occurs every year on broad bean in the southern part of the Province, but seldom appears in the northern part except on late crops of beans.

In Yunnan, a dry and a wet season occur every year. Roughly speaking, the dry season lasts 7 months, from the middle of October to the first part of June. It is in this dry season that broad beans are grown. In the southern part of the Province the crops are generally harvested between the end of March and the first part of May, while in the north the harvesting is one to one and a half months later. It is especially dry from December to April. The average monthly precipitation throughout this period seldom exceeds 70 mm. in almost all the bean-growing regions. There is, however, wide variation between day and night temperatures which brings on a regular dew formation that favors infection and the establishment of the mildew organism. Apparently, humidity is not the factor that will limit the occurrence of the disease in all of these bean-growing regions.

The air temperatures in the different regions are, on the other hand, extremely variable according to the elevation as well as the latitude. Studies, surveys, and observations in 1940, and subsequent years, have demonstrated a correlation between air temperature and the occurrence of the disease. As shown in table 1, powdery mildew was observed early in February at Kai-yuen. At that time, the average monthly air temperature was 20.4° C. In Chengkiang and Tsu-hsiun, diseased beans were found in April, when the average air temperatures were, respectively, 21.7° and 21.4° C. At Tai-li and Kunming, however, where average air temperatures usually were below

TABLE 1.—*Occurrence of powdery mildew of broad bean in relation to air temperatures at five localities in Yunnan in 1940*

Locality	Month in which disease was observed	Average monthly air temperature in degrees centigrade					
		Jan.	Feb.	Mar.	Apr.	May	June
Kai-yuen	Feb.-March	20.5	20.5	22.4	22.3	23.8	23.2
Chengkiang	March-April	14.7	14.7	19.1	21.7	25.8	25.3
Tsu-hsiun	March-April	19.2	19.4	20.3	21.4	22.2	23.1
Kunming	May-July*	9.8	11.3	14.2	18.0	19.5	19.6
Tai-li	May-July*	10.7	11.4	16.1	18.2	19.4	21.7

* On volunteer bean plants left in the field.

20° C., disease seldom appeared on the regular crop but was seen from May to July on volunteer plants left in the field or on certain late crops.

An experiment was made in the winter of 1942 to determine the relation of air temperature to the development of the disease. Young stems bearing a few healthy leaves were detached from bean plants, inoculated with conidia, and placed with bases in small flasks of water in chambers at different temperatures. At the end of three days, conspicuous mycelium of the fungus was seen on the leaves held at 20° C. to 24° C. Those held at 18° C. or below produced slight or no mycelium at all during the 7 days of the experiment. Although the experiments were preliminary, the results obtained seem to substantiate the observations made in the field that a relatively high air temperature during the spring favors the development of the disease in Yunnan.

HOST RANGE AND PHYSIOLOGIC SPECIALIZATION

It has been demonstrated by Salmon (5), Hammerlund (3), Blumer (1, 2), Searle (6), and others that *Erysiphe polygoni* DC. em. Salm. comprises a large number of physiologic races that differ from one another in pathogenicity. In most cases, each race is confined to a single species or to a few closely related species of the host and seldom has power to infect other genera.

Hammerlund (3) and Blumer (1, 2) independently investigated the specialization of parasitism in *Erysiphe polygoni* DC. Hammerlund distinguished 26 "form species" of this fungus. Among them, the following are found on the leguminous plants: f. sp. *medicagensis-sativae* on *Medicago sativa* and *M. falcata*; f. sp. *meliloti* on three species of *Melilotus*; f. sp. *trifolii repentis* on *Trifolium repens*; f. sp. *trifolii pratensis* on *Trifolium pratense* and *T. medium*; f. sp. *vicia-sativae* on *Vicia sativa* and other species of *Vicia*; f. sp. *pisi* on *Pisum sativum* and *P. arvense*.

Blumer (2) divided *Erysiphe polygoni* DC. em. Salm. into several species. Of these, *Erysiphe pisi* DC. attacks species of *Pisum*, *Doryenium*, *Medicago*, *Lupinus*, and *Vicia* among the Leguminosae. According to his own investigation and the infection experiments of both Salmon and Hammerlund, Blumer set up 4 "form species" in *Erysiphe pisi* DC.: f. sp. *pisi* Hammerlund on *Pisum sativum*; f. sp. *medicaginis sativae* Hammerlund on *Medicago sativa* and *M. falcata*; f. sp. *medicaginis lupulinae* Hammerlund on *Medicago lupulina*; and f. sp. *viciae sativa* Hammerlund on *Vicia sativa*, *V. sepium* and *V. silvatica*. Blumer stated that there might exist another "form species" on the genus *Vicia* although the specialization of its parasitism was not then clear. Searle (6), early in 1920, studied physiologic specialization in *Erysiphe polygoni* DC. on certain kinds of host plants. He showed that the fungi on *Polygonum aviculare*, *Trifolium pratense*, and *Pisum sativum* are distinct physiologic races. In one series of his inoculation experiments, conidia from *Pisum sativum* were used to inoculate *Pisum sativum*, *Onobrychis sativa*, *Vicia faba*, and *Trifolium pratense*. His results

indicated that conidia from *Pisum sativum* were unable to infect any of these plants; the fungus is said to be specialized on its own host, *P. sativum*. This confirms the fact, already noted by Salmon (5), that *Erysiphe polygoni* DC. on *Pisum sativum* is a distinct physiologic race and is unable to cause infection on *Vicia faba*.

From these investigations, it is known that *Erysiphe polygoni* DC. on *Pisum sativum* is a physiologic race distinct from the race attacking species of *Vicia*, while the *Erysiphe polygoni* on the different species of *Vicia* probably comprise a single race.

On account of the favorable weather conditions, *Erysiphe polygoni* DC. has been observed on a number of different host plants in this part of China. Material was available, therefore, for cross-inoculation experiments with the fungi collected from the various Leguminosae.

The first series of inoculations was with conidia from *Pisum sativum*,

TABLE 2.—Cross-inoculation experiments with conidia from *Pisum sativum*, *Vicia faba*, and *Lathyrus quinquenervius* in 1941 and 1942

Conidia from	Host inoculated					
	<i>Pisum sativum</i>		<i>Vicia faba</i>		<i>Lathyrus quinquenervius</i>	
	No. inoculated	No. infected	No. inoculated	No. infected	No. inoculated	No. infected
<i>Pisum sativum</i>	16	16 + +	20	18 + -	12	12 (0)
<i>Vicia faba</i>	20	19 + +	16	14 + -	15	15 (0)
<i>Lathyrus quinquenervius</i>	6	6 (0)	7	7 (0)	10	10 + -

Vicia faba, and *Lathyrus quinquenervius*. In order to avoid contamination, the tests were made in separate greenhouses. The method of inoculation was the same as described. In table 2 the + + sign denotes abundant mycelium accompanied by abundant sporulation, + - denotes trace to moderate mycelium accompanied by red spots or lesions, and the 0 denotes no macroscopically evident mycelium.

The results indicate, contrary to the results obtained by the previous investigators, that the fungus from *Pisum sativum* is able to cause infection on both *Pisum sativum* and *Vicia faba*, and the same is true for the fungus from *Vicia faba*. Evidently, the two should be regarded as the same physiologic race, whereas that from *Lathyrus quinquenervius* is a distinct race.

In examining the types of infection produced by conidia from either *Pisum sativum* or *Vicia faba*, it was noted that, in most cases, the mycelium was much scantier on *Vicia faba* than on *Pisum sativum*. Inasmuch as the identity of the two cultures has been proved by cross-inoculation experiments, it is suggested that the difference in degree of infection on the two hosts was probably due to a difference in the nature of host plants rather than in the fungi themselves. In other words, the beans are more resistant to the attack of the fungus than the peas.

The second series of cross inoculations was conducted with an attempt to determine the pathogenicity of *Erysiphe polygoni* DC. collected from the various species of *Vicia*, including *Vicia cracca*, *V. faba*, *V. hirsuta*, *V. sepium*, and *V. tetrasperma*, all of which are commonly mildewed in Kunming and its vicinity. In addition, seeds of many other species of *Vicia* were obtained through the kindness of the United States Department of Agriculture, Kew Gardens in England, and various institutes in China. These seeds were sown in small plots each fall since 1940. *Erysiphe polygoni* DC. has been found on all of them and material was collected from these plots for cross inoculations.

In the winter of 1940, 1941, and 1942, healthy seedlings of *Vicia angustifolia*, *V. atropurpurea*, *V. cracca*, *V. dasycarpa*, *V. faba*, *V. gigantea*, *V. hirsuta*, *V. leucantha*, *V. lutea*, *V. monantha*, *V. orobus*, *V. pannonica*, *V. sativa*, *V. sepium*, *V. tetrasperma*, *V. tridentata*, and *V. villosa* were raised in a clean greenhouse. Cross inoculations were made to potted plants in other greenhouses and were repeated to insure the development of the disease. In order to avoid contamination, the inoculated seedlings were left in large glass chimneys covered with a thin layer of cotton at the top. The results of these experiments indicated that conidia taken from any of these species of *Vicia* were capable of infecting all of the other species used. These cultures are, therefore, regarded as a single physiologic race of *Erysiphe polygoni* DC. and are considered to be the same race that has been found on *Pisum sativum* in this part of China.

The reactions of these *Vicia* host plants to the attack of the fungus were by no means alike in these experiments. Many of the species seemed more susceptible to the disease than the others. For example, on *Vicia orobus* the fungus usually produced an abundant mycelium and abundant spores, and it rarely formed red lesions as it did on *V. faba*.

The term physiologic race is generally used to refer to strains of the fungus which are morphologically indistinguishable. However, in certain species of *Erysiphe* the so-called physiologic races can be differentiated from one another by the morphology of either the conidia or the perithecia. Blumer (2), for instance, has distinguished *Erysiphe pisi* on *Medicago*, *Pisum*, and *Vicia* by the size of perithecia. Homma (4) has made a comparative study, based on the difference in conidial dimensions, of the various physiologic races of *Erysiphe graminis* DC. and has found that there are appreciable morphological differences between the races on common wheat, naked barley, *Poa annua*, and *Elymus mollis*. Yarwood (8) has also shown that the forms of *Erysiphe polygoni* DC. on bean, red clover, California poppy, mustard, pea, cowpea, buckwheat, and Columbine can be distinguished readily from each other by the gross morphology of their conidiophores and conidia.

One hundred viable conidia of the mildew fungus from each of the various species of *Vicia*, and from *Pisum sativum* were measured while mounted in water under cover slips (Table 3).

No decided constant differences were noted in the size and shape of conidia taken from any of these host plants. This is further evidence that the powdery mildew of *Vicia* species and of *Pisum sativum* in Yunnan is caused by the same physiologic race of *Erysiphe polygoni* DC.

Since the fungus is able to infect both peas and broad beans in China, then to which "form species," viz. *pisi* or *viciae sativa* given by Blumer is it more related? Indeed, in the infection experiments, the peas were more susceptible to the attack of the fungus than the beans. However, many other species of *Vicia*, such as *V. orobus* and *V. gigantea* were equally, if not more, susceptible to the disease than peas. Evidently, the difference in degree of susceptibility of the hosts has nothing to do with the question. In case of morphologic difference, Blumer (2) distinguished the form species of *Erysiphe pisi* DC. on *Medicago sativa*, *Vicia sativa*, and *Pisum sativum* by

TABLE 3.—Measurements of conidia of *Erysiphe polygoni* DC. from naturally infected *Vicia* species and from *Pisum sativum*

Host	Range for conidia, in μ		Average, in μ
	Length	Width	
<i>Vicia atropurpurea</i>	27.8–45.3	13.9–20.9	36.7 \times 17.4
<i>V. cracca</i>	29.6–48.7	13.9–20.9	37.8 \times 17.0
<i>V. dasycarpa</i>	33.1–17.0	15.7–20.9	39.7 \times 18.4
<i>V. faba</i>	27.9–47.0	12.2–22.6	38.3 \times 19.1
<i>V. gigantea</i>	27.7–43.5	13.9–20.9	37.6 \times 17.4
<i>V. hirsuta</i>	29.6–47.0	13.9–20.9	37.9 \times 17.8
<i>V. lutea</i>	26.1–48.7	12.2–19.1	36.9 \times 17.2
<i>V. monantha</i>	29.6–43.5	15.7–22.6	36.7 \times 19.0
<i>V. orobus</i>	26.1–43.5	13.9–20.9	35.4 \times 16.9
<i>V. pannonica</i>	31.3–48.7	13.9–20.9	39.1 \times 18.1
<i>V. sativa</i>	27.9–45.6	13.3–22.6	36.5 \times 18.8
<i>V. sepium</i>	27.9–47.0	13.9–20.9	39.4 \times 17.6
<i>V. tetrasperma</i>	29.6–48.7	13.9–20.9	37.9 \times 16.8
<i>V. villosa</i>	27.9–48.7	13.9–20.9	35.7 \times 18.3
<i>Pisum sativum</i>	31.3–48.7	13.9–20.9	38.5 \times 18.6

the size of their respective perithecia. According to his measurements, the perithecia of the fungus on *Vicia* and on *Pisum* were similar: Perithecia from *Vicia* ranged from 97 to 119 μ in diameter with an average of 108.3 μ , and those from *Pisum* ranged from 102 to 126 μ with an average of 114.4 μ . The perithecia of the fungus on *Medicago sativa* were decidedly smaller, from 85 to 101 μ in diameter with an average of 93.2 μ . The perithecia measurements of the fungus in China, as given before, are closer to those of perithecia from *Vicia sativum* than those from *Pisum sativum* as given by Blumer. It is therefore justified to consider the fungus in China as a new form of *Erysiphe polygoni* DC. parasitizing both the species of *Vicia* and *Pisum*, and f. sp. *viciae pisi* is accordingly proposed in order to distinguish it from f. sp. *viciae sativae* Hammerlund (Blumer) which occurs only on the genus *Vicia*. The present race is also known to be distinct from f. sp. *pisi* Hammerlund (Blumer) by reason of its ability to infect *Vicia* in addition to infecting *Pisum*.

METHODS OF OVERWINTERING

Studies made by the writer during the past three years have shown that the powdery mildew of broad bean may overwinter either as conidia or perithecia on beans, peas, or wild species of *Vicia*.

Mature perithecia containing asci with ascospores were occasionally observed on *Vicia sativa* in the spring. No perithecia were found on beans and peas in nature.

In southern Yunnan, the winters are so mild that many wild species of *Vicia*, such as *Vicia sativa* and *V. hirsuta*, usually survive the winter. Conidia are found on them in the early spring.

It is also interesting to point out that in certain districts of southern Yunnan, two crops of beans are raised. The second crop, primarily for table use, is spring-sown and is harvested during November, December, and January of the next year. Conidia have been found on them even in the winter months. Evidently, this second crop serves as the source of infection for the fall-sown beans.

DISCUSSION

Field observations and laboratory experiments have revealed that the occurrence of *Erysiphe polygoni* DC. in Yunnan seems to be correlated with air temperatures. The disease rarely occurs in those regions where the average monthly air temperature during the later stages of plant development is below 20° C. However, it is surprising to find that powdery mildew of pea is not only coexistent with the crop but is also present in winter when the average air temperature rarely exceeds 16° C. in most of the pea-growing districts. Since the fungus on peas and beans constitutes a single physiologic race of *Erysiphe polygoni* DC., it is hard to give reasons why the air temperature will limit the development of the fungus on one kind of host and not on the other. It seems, therefore, that in the studies on the environmental factors in relation to the occurrence of a disease, the nature of the host plants should also be taken into consideration. This assumption, although a matter of speculation, is, nevertheless, of some interest.

SUMMARY

Powdery mildew of broad bean, caused by *Erysiphe polygoni* DC., is very prevalent in the bean-growing regions of southern Yunnan Province in China. Although it is of no great economic importance, it occasionally damages the crop.

Leaves, petioles, stems, and pods of plants are attacked. Only in severe cases, does mildew blight the stem tips and cause premature ripening of young pods.

In contradiction to the results obtained by previous investigators, the mildew fungus on broad beans is able to cause infection on *Pisum sativum*. Based on this finding, powdery mildews of broad beans and peas in Yunnan is considered to be caused by a single physiologic race and is herein design-

nated as *Erysiphe polygoni* DC. *viciae pisi* forma nova., a race which differs from f. sp. *vicia-sativae* Hammerlund by its ability to infect *Pisum sativum*. Powdery mildew on *Lathyrus quinquenervius* is a distinct race.

The occurrence of the disease is correlated with relatively high air temperatures during March, April, and May. An average monthly temperature of 20° C. or above favors the development of the disease on broad bean.

The fungus is capable of overwintering as either conidia or perithecia on beans and peas as well as on the wild species of *Vicia*.

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PHYTOPATHOLOGICAL NOTES

Perithecia of Powdery Mildew on Zinnia Seed.—There are few records of the occurrence or transmission of powdery mildews on seeds. *Erysiphe polygoni* DC. has been reported as externally and internally borne by pea seed^{1,2,3} and externally carried on seed of *Cuminum cyminum*.⁴ Circumstantial evidence has been presented⁵ that *E. cichoracearum* DC. was carried on cineraria seed, and it was suspected⁶ that *E. Fischeri* Blumer on *Senecio* spp. and *E. horridula* (Wallr.) Lév. on *Myosotis* were transmitted on seed.

In several commercial zinnia seed fields in Ventura and Santa Barbara Counties, California, in which powdery mildew had not been controlled effectively, the fungus, *E. cichoracearum*, developed copiously in the flower heads and there formed abundant perithecia in October, 1943, particularly on the

TABLE 1.—Incidence of perithecia of *Erysiphe cichoracearum* on zinnia seed hand-picked and threshed by hand or machinery (1943)

Variety	Lot number and handling of seed	Number seeds examined	Percentage seeds	
			With 10–100 perithecia	With 1–9 perithecia
Oriole	1. Picked in September; hand-threshed	488	2.66	11.68
do	2. Picked in October; hand-threshed	4,690	1.07	5.57
do	3. Picked in October; hand-threshed	3,034	1.42	5.11
do	4. Picked in October; commercially threshed and cleaned	9,253	0.34	1.97
Mixed	5. Picked in September; hand-threshed	3,396	0.29	1.71
Total or average		20,861	0.70	3.42

variety Giant Dahlia Flowered Oriole. Perithecia were most abundant at the stylar end of the seed and usually were formed at the lower edge of petal infections which extended onto the immature seed (achene). The cup formed at the stylar end of zinnia seed by the petal base provides a protected cavity in which microorganisms can be carried.

The incidence of perithecia on zinnia seeds handled in various ways is shown in table 1. Lots 1, 2, 3, and 5 were carefully picked and the chaff removed by hand in the laboratory. Lot 4, commercially hand-picked, dried on canvas, machine-threshed, and machine-cleaned, still carried perithecia on 2.31 per cent of the seed.

¹ Van Hook, J. M. Powdery mildew of the pea. Ohio Agr. Exp. Sta. Bull. 173: 247–249. 1906.

² Crawford, R. F. Powdery mildew of peas. New Mexico Agr. Exp. Sta. Bull. 163. 1927.

³ Uppal, B. N., M. K. Patel, and M. N. Kamat. Pea powdery mildew in Bombay. Bombay Dept. Agr. Bull. 177. 1935.

⁴ Uppal, B. N., and M. K. Desai. Cumin powdery mildew in Bombay. Bombay Dept. Agr. Bull. 169. 1933.

⁵ Macdonald, J. A. Powdery mildew on cinerarias. Gard. Chron. 105(2721): 111. 1939.

⁶ Blumer, S. Die Erysiphaceen Mitteleuropas mit besonderer Berücksichtigung der Schweiz. In Beiträge zur Kryptogamenflora der Schweiz 7(1): 46. 1933.

Samples of these lots were grown in flats of sterilized soil in the greenhouse for 6 weeks the following spring, but no mildew ever developed on the plants. Perithecia were still present, although in reduced numbers, on the seed coats after they had been pushed through the soil in germination. Apparently the ascospores were not released or, if any were, did not infect. This is not surprising in view of the difficulties which have been reported by various workers in germination of ascospores of powdery mildews. However, it is possible that infection rarely occurs from perithecia of powdery mildew on zinnia seed.

Powdery mildew perithecia on seeds such as zinnia, which have epigeal germination, would be in a much more favorable position for producing ascospore infection than those on seeds such as pea, which have hypogeal germination. The internal infection described for pea seed would, of course, represent a different phenomenon.

Powdery mildew is relatively easy to control in commercial zinnia seed fields in California by frequent and consistent dusting with sulphur unless infections become established in the flower heads. Such infections may greatly reduce yield and quality of seed, as well as lead to the possibility of seed transmission. Emphasis on control early in the season to prevent flower infection is indicated.—KENNETH F. BAKER and WARREN F. LOCKE, Division of Plant Pathology, University of California, Los Angeles.

Camellia Flower Blight.—In 1940, a camellia flower blight was described as new¹ and the causal fungus was designated *Sclerotinia Camelliae*. Professor Iwao Hino of Miyazaki College of Agriculture later called to our attention the fact that Mr. K. Hara had described^{2, 3} what appeared to be the same disease and fungus in Japan about twenty years earlier. While Hara's dimensions for asci ($120-140 \times 6-8 \mu$) and ascospores ($8-11 \times 4-5 \mu$) are somewhat larger than ours ($100-125 \times 4.3-5.8$ and $5.3-7 \times 2.5-3.5$), it appears that the fungus here is essentially the same as that in Japan and should be designated hereafter as *S. Camelliae* Hara.

It is probable moreover that the fungus was introduced into the United States from Japan as suggested in our earlier report¹ in view of the above additional evidence and the further fact that the disease has since been found in California in a second nursery which imported plants from Japan.

All told the disease has been found in several other nurseries and parks and in a few private gardens in California. Apparently it is not definitely known to be established elsewhere in the United States.

Although considerable effort was expended in removal and destruction of affected flowers in the nursery where the disease was first seen in 1938, there were numerous infected flowers in 1943 and 1944. This nursery com-

¹ Hansen, H. N., and H. Earl Thomas. Flower blight of camellias. *Phytopath.* 30: 166-170. 1940.

² Hara, Kanesuke. [On a *Sclerotinia* disease of *Camellia*.] *Dainippon Sanrinkwaihō* No. 436. pp. 29-31. 1919. (In Japanese.)

³ Hara, Kanesuke. [Sclerotinia disease of *Camellia*.] *Zikken Zyunmoku Byōgihen*. pp. 251-252. 1927.

prises several acres and the problem of eradicating the disease is further complicated by the practice of maintaining numerous plants in containers of assorted sizes and shapes, which may afford concealment for some of the sclerotia. However, in 1944 the nursery, in addition to destroying infected flowers, began ground-spraying with Fermate. Whether the spraying contributed to the result is not yet clear. At any rate the disease in 1945 was limited to two small areas, the largest only a few yards in diameter. A preliminary test on some of the buried sclerotia, indicated that Fermate at 4 lb. in 100 gallons did not kill mature apothecia but seemed to inhibit formation of additional apothecia.

Since wholesale disbudding for one or more years was under consideration as an alternative control measure, some information on the interval between sclerotium formation and the production of apothecia was needed. Accordingly in April, 1943, whole blighted flowers and petals separated from such flowers were placed on soil in boxes and covered about one inch deep by peat moss and placed under lath. Sclerotia collected in 1938 and kept dry until the beginning of this experiment were similarly buried. The moss was kept continuously wet thereafter. On February 24, 1944, apothecia were found in the box containing whole flowers and in the box in which petals had been buried. No apothecia arose from the old sclerotia at that time or afterward. Apothecia were produced in greater numbers and over a longer period from the whole flowers than from separate petals, the last apothecia for 1944 appearing about mid-April. A few sclerotia from whole flowers were removed and examined on May 13, 1944, and found to be firm, although one of them had produced several apothecia. The remainder were left undisturbed until the spring of 1945, the medium having been kept moist continuously. The first apothecia were found on February 6, 1945, in the box with whole flowers and the last⁴ about April 15. No apothecia appeared in 1945 in the box containing petals only.

In an attempt to determine whether the fungus is able to invade unopened flowers, several large plants with numerous flower buds were placed under glass at a distance from the nursery. All buds with petals exposed at the time the plants were moved were marked at that time, and only those buds developed symptoms of the disease.

Since only the ascospores are infective and only flowers are infected, it should be possible to eliminate the fungus altogether by systematic destruction of infected flowers possibly supplemented by ground spraying.

In the meantime discretion should be observed in the movement of balled or boxed plants from infested to noninfested areas.—H. EARL THOMAS and H. N. HANSEN, Division of Plant Pathology, University of California, Berkeley, California.

Yellow Berry and Stem Rust of Wheat.—Under Palestinian conditions only a few varieties of imported vulgare wheats (CCC, BIPM, Giza 7) bear starchy kernels. Almost all indigenous durum and imported durum and

⁴ While this note was in press apothecia appeared again early in February, 1946.

vulgare wheats bear normally characteristic flinty kernels. However, not infrequently, kernels affected with yellow spots may be found among the flinty kernels. This phenomenon is characteristic for either durum varieties moderately resistant to rust, or vulgare varieties that usually escape rust, both under conditions of rust attack.

In field trials with the control of wheat rusts by sulphur dusts,¹ it was observed that treated plants yielded only 0.2 per cent kernels affected with yellow spots, while the nondusted rusted plants yielded from 5 to 63 per cent, the average of such affected grains being 24 per cent. It is apparent, therefore, that yellow spotted kernels may be connected with rust attack of plants.

The analysis of kernels from rusted plants revealed a lower weight, lower protein and gluten content, and an inferior gluten and baking quality as compared with kernels from plants dusted with sulphur.²

Yellow berry of wheat has been described since 1904 as a physiogenic disease. No mention is made anywhere of the influence of rust attack in the enumeration of the causes of yellow berry. Studies revealed abnormal physical and chemical constants and inferior baking quality of diseased kernels. Heald^{3, 4} reviewing the literature dealing with yellow berry summarizes the possible causes influencing the appearance of yellow berry as follows: (1) climatic factors operating upon the grain while in chaff, either during the last part of the ripening period or after cutting; (2) hereditary tendencies operating independent of environment; (3) disturbed nutrition, due to unfavorable water or soil relations.

The trial mentioned was made in a field under uniform cultivation and fertilization, sown with a pure selection of Florence wheat (Morocco origin). This is a mid-early vulgare variety bearing flinty kernels. It escapes rust attack when sown early but is very susceptible to stem rust when sown late. The dusted plants were mature and harvested ten days earlier than the nondusted ones.

Other factors being the same, the kernels affected with yellow spots in this trial may be ascribed to the influence of stem rust. Late sowing and subsequent late maturity influencing the appearance of yellow berry, as mentioned by Heald, may be considered as an indirect cause of the disease, particularly in wheat varieties escaping rust. It is suggested that decrease of protein content in affected kernels may be influenced by rust.

Since the application of sulphur, which controlled rust, also checked yellow berry, it may be assumed that the yellow berry is etiologically connected with the rust attack.

It may be supposed, therefore, that yellow berry in wheat bearing normally flinty kernels also may be controlled when sulphur is applied for the

¹ Minz, G. Control of rust in wheat by sulphur dusting. *Hassadeh* 25(3-4): 107. 1945. (Hebrew.)

² Plaut, M. Chemical analysis and baking test of flour from rusted wheat. Unpublished data.

³ Heald, F. D. *Manual of plant diseases*. 953 pp. 1933.

⁴ Heald, F. D. *Introduction to plant pathology*. 603 pp. 1943.

control of rusts in wheat.—GERSHON MINZ, Division of Plant Pathology, Agricultural Research Station, Rehovot, Palestine.

*Physiologic Races of Yellow Rust of Raspberries in Western Washington.*¹—For many years yellow rust, *Phragmidium rubi-idaei* (DC.) Karst., has been an economic factor in the culture of red raspberries of western Washington. This disease has contributed to the decline of the Cuthbert and Marlboro varieties which are rapidly being replaced by the Washington and Tahoma varieties in this region. After the new Washington variety was introduced² it was observed to remain free from infection by yellow rust in the Puyallup Valley, even when grown close to rust-infected Cuthberts. These observations indicated that Washington might be immune or highly resistant to infection by *P. rubi-idaei*. However, in July, 1944, leaf specimens of Washington affected with yellow rust were sent to the writer by the Agricultural Extension Agent of Whatcom County, 125 miles north of the Puyallup Valley. Field observations showed rust to be generally present from a moderate to a severe degree in many commercial fields in Whatcom County where Washington was grown. Both uredial and telial stages were on the leaves of fruiting canes and current season's growth.

Three young plants, about two feet tall, of both Washington and Cuthbert were inoculated in the greenhouse at Puyallup, with a water suspension of urediospores from specimens of the rust brought in sealed bags from Whatcom County. Infection of both varieties was about the same (Fig. 1, A and B). The uredia were large and numerous on the leaves of all inoculated plants. A similar test was made with urediospores collected from the Marlboro and Cuthbert varieties grown in the Puyallup Valley. The Cuthbert plants were severely infected, but those of Washington appeared to be resistant (Fig. 1, C and D). Uredia, surrounded by more or less chlorotic areas, were present on the leaves of the latter variety but they were exceedingly small and never fully developed in comparison to those on the Cuthbert leaves. These tests were repeated in the greenhouse on six different occasions during the winter of 1944, with similar results. In two subsequent experiments the Tahoma variety was inoculated with the rust from both the Puyallup Valley and Whatcom County but no sori were observed on the inoculated leaves. Cuthberts serving as a check were heavily infected.

In raspberry fields in Whatcom County inspected during the summer of 1944 and 1945 there was no rust on Lloyd George or Newburgh varieties grown adjacent to affected Washington raspberries. In the Puyallup Valley the Cuthbert and Marlboro varieties were affected but only an occasional sorus was found on Washington. In instances where Cuthbert and Washington plants were grown in adjacent rows, or where Washington was interplanted in Cuthbert rows, Cuthbert was infected with rust, while no sori were observed on Washington. No rust infections have been observed on

¹ Published as Scientific Paper No. 656, College of Agriculture and Agricultural Experiment Stations, State College of Washington.

² Schwartz, C. D. The Washington and Tahoma red raspberries. Wash. Agr. Exp. Stat. Pop. Bull. No. 153. 1938.

Tahoma plants. Thus far Washington has been found affected with rust as far south as Snohomish County, but no serious outbreaks of the disease have been seen or reported on this variety southward to the Columbia River. On the other hand, Cuthbert and Marlboro, of which there remain only a few fields, are affected to some extent each year in this area.

The results of these experiments and observations suggest the likelihood that there are two distinct physiologic races of *Phragmidium rubi-idaei* in western Washington. Both Cuthbert and Washington raspberries are susceptible to the rust present on the latter variety in Whatcom County, while

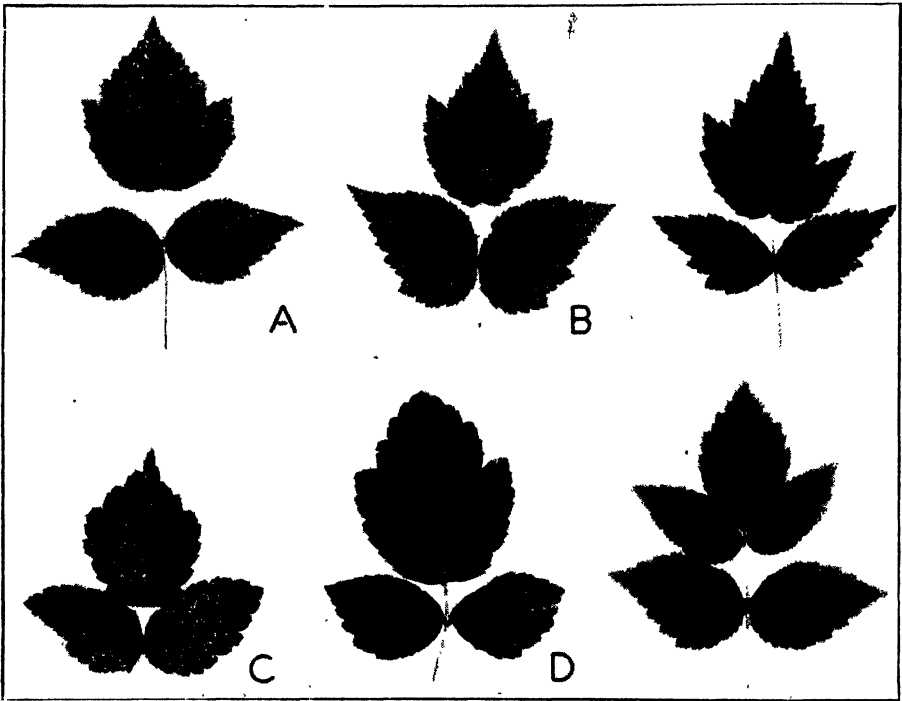


FIG. 1. A, B. Washington and Cuthbert raspberry leaves, respectively, with sori of western yellow rust after inoculation with rust collected from Washington variety in Whatcom County. Healthy Cuthbert leaf at right. C, D. Cuthbert and Washington leaves inoculated with rust collected from Cuthbert and Marlboro raspberries in Puyallup Valley. Note the scattered undeveloped sori on the Washington variety, D. Healthy Washington leaf at right.

Washington is resistant to the race collected from Marlboro and Cuthbert in the Puyallup Valley. In this case it seems unlikely that climatic factors might determine to such a large extent the development of the disease on different varieties, as has been referred to by Zeller.³ He found that in Oregon the variety Ranere was more resistant to *P. rubi-idaei* than Cuthbert, but that the reverse is true in coastal regions of California.—FOLKE JOHNSON Washington Agricultural Experiment Stations, Western Washington Experiment Station, Puyallup, Washington.

³ Zeller, S. M., and W. T. Lund. Yellow rust of Rubus. Phytopath. 24: 257-265.

*The Relation of Stomatal Behavior at the Time of Inoculation to the Severity of Infection of Soybeans by Xanthomonas phaseoli var. sojense (Hedges) (Starr) Burk.*¹—Difficulty in obtaining consistently good infection with bacterial leaf spots of field-grown crops has been a common experience. The testing of varieties for disease resistance requires reliable methods in order to be certain of the interpretation of the results. Stomatal behavior has been shown by Diachun² to be of importance in the infection of tobacco leaves by bacteria. Inoculation experiments were conducted in 1945 to evaluate this factor in the infection of soybeans in the field with the bacterial pustule disease caused by *Xanthomonas phaseoli* var. *sojense* (Hedges) (Starr) Burk. The experiments were performed at Columbia, Missouri, and Urbana, Illinois.

Different plots of the soybean varieties Lincoln and Chief were inoculated at two-hour intervals throughout one day in each of the tests. The inoculation started at 3 a.m. Central Standard Time when the stomata were practically closed and continued until 9 p.m. when the stomata were again practically closed. Strips of the lower leaf epidermis were placed into 100 per cent ethyl alcohol at the time of each inoculation and later the average width of the stomatal openings was determined. At Urbana, the light intensity was measured at the time of each inoculation. Precautions were taken to provide uniform inoculum throughout the day. The inoculum was applied with a power sprayer, which maintained approximately 150 pounds pressure, with the nozzle adjusted to deliver a stream of water unbroken until within a few inches of the leaves where it broke up into large droplets similar to beating rain. In each of the three tests with Lincoln and Chief, four replications were arranged as randomized blocks. Disease notes were taken ten to fourteen days after inoculation. The severity of infection for a row was rated on an arbitrary scale of 0 to 10, no infection being indicated by 0, and heavy infection on all leaves being indicated by 10.

The results have shown that inoculation between 8 a.m. and 2 p.m. gives the greatest amount of infection (Fig. 1). A logical conclusion would be that the bacteria enter when the stomata are open. Considerable dew developed on the plants during the nights concerned in each test, and attempts were made to select days of normal sunshine. This was nearly accomplished at Urbana, but in the tests at Columbia, it was partly cloudy during part of the day. Although the effect of cloudiness on stomatal behavior was not marked, it may be reflected slightly in the difference in steepness of the curves in figure 1 for the two locations. At Columbia, where slight cloudiness existed, the effect of increase in light between 6 a.m. and 8 a.m. was

¹ A contribution from the U. S. Regional Soybean Laboratory, a cooperative organization participated in by the Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Agricultural Experiment Stations of Alabama, Arkansas, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Louisiana, Michigan, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, North Dakota, Ohio, Oklahoma, South Carolina, South Dakota, Tennessee, Texas, Virginia, and Wisconsin. Missouri College of Agriculture Journal Series No. 990.

² Diachun, Stephen. Relation of stomata to infection of tobacco leaves by *Bacterium tabacum*. Phytopath. 30: 263-272. 1940.

not as sharp as at Urbana. Watersoaking of the leaf tissue by the spray was apparent immediately after inoculation during the bright part of the day, but was inconspicuous after inoculation at other times.

The results of these tests indicate that stomatal behavior should be a prime factor in the timing of inoculation of soybeans. Varieties which appear to be resistant when inoculated at midday, however, should be further examined to be certain that they do not have a different diurnal cycle, thereby escaping inoculation at a time when they are vulnerable. The general tendency in the past has been to inoculate field plants in darker periods (*e.g.*, early in the morning) and without particular attention to the force of the inoculum hitting the leaves.

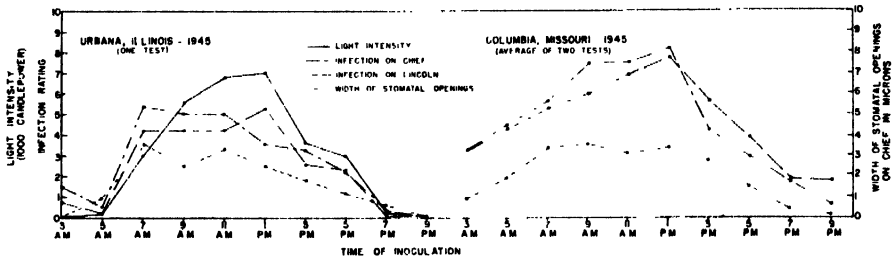


FIG. 1. Graph showing the correlation of stomatal behavior and infection ratings on Chief and Lincoln soybeans at Urbana, Illinois, and Columbia, Missouri, when inoculated by power spraying at different times of the day. (Central Standard Time.)

It is believed that the results reported here will be of considerable value in the development of an improved technique for inducing artificial epidemics in the field.—WILLIAM B. ALLINGTON, Associate Pathologist, U. S. Regional Soybean Laboratory, and CARL V. FEASTER, Cooperative Agent, Missouri Agricultural Experiment Station.

Reaction of Dodders to Stems of Other Dodders and to Their Own Stems.—While studying the relationship of the two dodders, *Cuscuta subinclusa* Dnr. and Hilg. and *C. californica* Choisy, to certain host plants commonly used in the study of curly top and other virus diseases, the question arose as to what occurs when the stems of these dodders coil around each other. When these united stems are sectioned, stained, and examined under the microscope it becomes apparent that haustoria penetrate the encircled stems and connect with the vascular bundles in the same way they do in the case of sugar-beet petioles. *C. californica*, for example, will parasitize *C. subinclusa*, *C. campestris* Yunecker and *C. americana* L.

Figure 1, A, shows *Cuscuta californica* growing on *C. americana* which in turn is parasitic on *Nicotiana glauca*. The small amount of vegetative growth and the abundance of flowers indicate that *C. americana* is not a favorable host. Figure 1, B, is a transverse section of a stem of *C. californica* with a haustorium of *C. subinclusa* grown into it, with xylem vessels of the invading haustorium connecting with similar tissues of *C. californica*.

Further proof that this dodder definitely parasitizes the others was

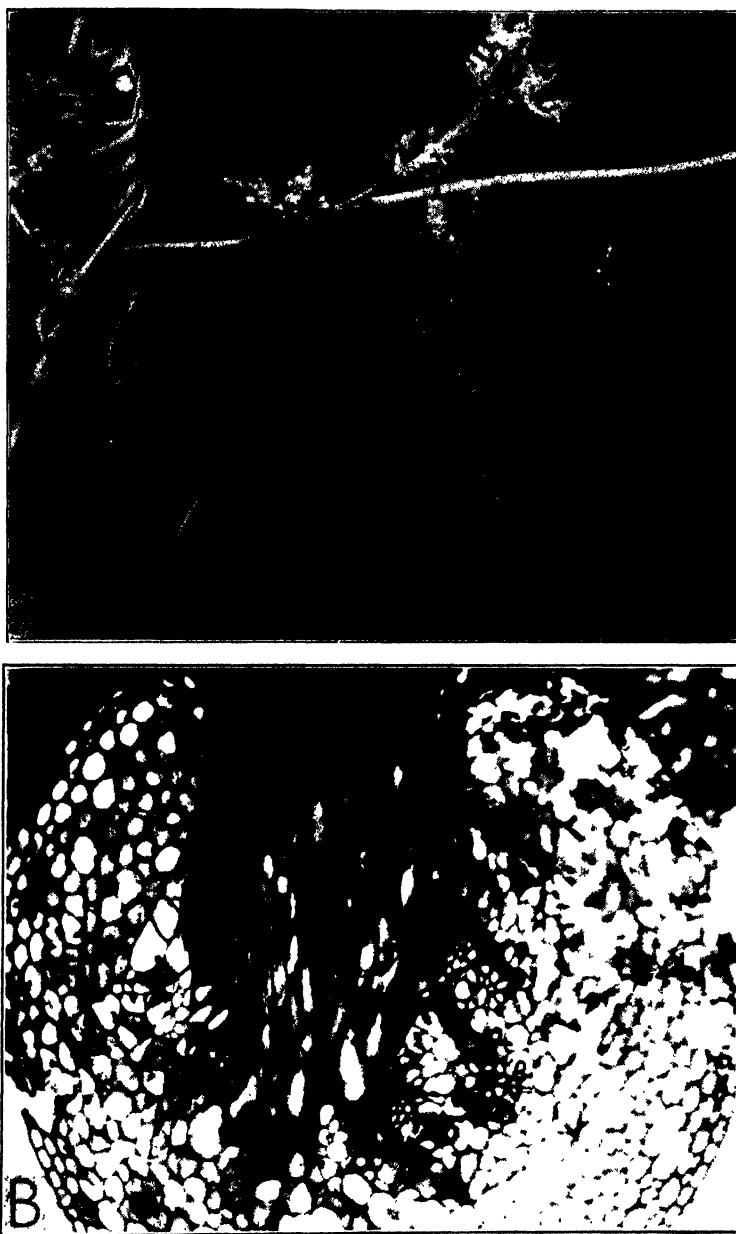


FIG. 1. A. *Cuscuta californica* growing on *C. americana*, parasitic on *Nicotiana glauca*. B. Transverse section of stem of *C. californica* invaded by haustorium of *C. subinclusa*, xylem elements of haustorium (a) connecting with similar elements of host (b).

obtained by the following tests. Rapidly growing stems of *C. californica* were taken from a tobacco plant and the basal ends put in a bottle of water. These stems were then allowed to establish themselves on the other species of dodders mentioned, which were growing on green host plants. Then these

stems were taken out of water and were forced to live on these dodder species as host plants.

The two dodders, *Cuscuta californica* and *C. subinclusa*, will turn back on their own stems and invade them with haustoria as they do on other species of dodder. There is no apparent harm done to the tissues of the dodders after being thus invaded. As in other host plants attacked by the dodders, the tissues are completely dissolved in the region occupied by the haustoria. Cells in contact with haustoria appear to be normal with no sign of disorganization. The haustorial hyphae contact phloem cells but do not invade them. They apparently receive nourishment from these host cells by diffusion. The xylem elements of the dodder intermingle with those of the host and seem to unite with them.—C. F. LACKEY, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, Riverside, California.

*Infection Studies with Actinomyces scabies.*¹—The pathogenicity of *Actinomyces scabies* (Thaxt.) Güssow, the causal organism of potato scab, is not well understood although its parasitism was demonstrated over a half century ago by Thaxter. In recent infection studies, the organism killed radish seedlings growing in sterilized, artificially infested, highly calcareous peat soil. A localized necrosis of potato leaf blades and petioles has been obtained after spore suspensions were applied to wounded tissues.

Radish seedlings have been killed by the scab organism under controlled conditions in the laboratory. Isolates of *Actinomyces scabies* obtained from scab lesions on potato and red beet and known to be extremely virulent to potato were used throughout. Seedlings which had been surface disinfected and germinated on 2 per cent water agar were placed in large, Pyrex test tubes containing sterilized peat soil which had been artificially infested with pure cultures of the scab organism. The first evidence of infection was on root tissue which became water-soaked with only slight discoloration. As necrosis developed, the tissues became dark brown. Aerial symptoms, developing first on the cotyledon, consisted of a wilting of the terminal portion of the leaf accompanied by general chlorosis and followed by necrosis of the entire lamina. The hypocotyl remained erect and apparently unaffected until after the terminal leaves were dead. Radish plants lived only 2 to 3 weeks in soil infested with *A. scabies*, whereas control plants growing in sterilized, noninfested soil were apparently free from disease and remained thus until the tests were terminated approximately one week later. Seedlings of radish grown in pots containing soil infested with the scab organism were noticeably stunted. Preliminary tests with cucumber, pepper, turnip, and spinach have indicated that *A. scabies* is sufficiently pathogenic to these plants to cause stunting and premature death. KenKnight² has made a detailed sur-

¹ Journal paper number J-1325 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 816.

² KenKnight, Glenn. Studies on soil actinomycetes in relation to potato scab and its control. Mich. Agr. Exp. Stat. Tech. Bull. 178. 1941.

vey of the literature concerning the development of *A. scabies* on the roots of various vegetables and weeds. He reported chiefly the production of localized, corky, scab-like lesions typical of those found on the tubers of the potato.

In our experiments, superficial, scale-like, necrotic areas, that were light gray to tan, were induced on Red Warba potato foliage by rubbing a spore suspension of the scab organism onto the leaves with the finger. Water-soaked spots which later dried and turned brown first appeared on the leaf blade and its veins. These necrotic areas did not enlarge after removal of the plants from the moist chamber 3 to 4 days after inoculation. Leaves of control plants rubbed with water in a similar manner, although evidencing some mechanical injury, did not become necrotic. Water-soaking the leaf tissues with a jet of spore suspension ejected from a hypodermic syringe onto the under surface of the leaf also resulted in necrotic lesions. When the plants were removed from the moist chamber, the water-soaked areas of plants treated with a spore suspension had a dark metallic luster, whereas the control leaves, treated with sterile water in a similar manner, were water-soaked and green. The affected tissues of the inoculated leaves soon became necrotic; the water-soaked areas of the control leaves recovered and no necrosis occurred. Necrotic areas of the inoculated leaves were brown to tan and apparently localized at the bases of trichomes. Affected areas did not noticeably enlarge after removal from the moist chamber. In general reactions were more severe on young leaves that were not completely expanded at the time of inoculation. In these preliminary trials, free-hand sections were not suitable for demonstrating actinomycetous filaments within affected tissues. Lutman² recently reported actinomycetous strands in apparently healthy leaves of potatoes.

Leaves situated at the soil line on Cobbler potato plants grown in artificially infested, peat soil have had a superficial necrosis similar to that described on leaves treated with suspensions of *Actinomyces scabies*. In a few instances necrosis was observed extending some distance into the petiole. The filaments of *A. scabies* were demonstrated in the intracellular spaces of such petioles by staining free-hand sections with Gentician violet.

Attempts to induce a reaction by atomizing spore suspensions on uninjured potato-leaf surfaces have not been successful.—W. J. HOOKER and G. C. KENT, Botany and Plant Pathology Section, Iowa Agricultural Experiment Station, Ames, Iowa.

Late Blight of Tomatoes in East Texas at Transplanting Time.—Tomato plants with late blight (caused by *Phytophthora infestans* (Mont.) de Bary) were found¹ near Jacksonville, Texas, in April, 1945. The late-blight symptoms appeared only on plants that had been brought from the Lower Rio

² Lutman, B. F. Actinomyceetes in various parts of the potato and other plants. Vt. Agr. Exp. Stat. Bull. 522. 1945.

¹ Altstatt, G. E. Tomato late blight in East Texas. U. S. Dept. Agr. Plant Dis. Rptr. 29: 344-345. 1945.

Grande Valley, or on locally grown plants that were near the fields where these imported plants were set. The plants were shipped in March from the Lower Valley where late blight was severe on tomatoes and potatoes,² and where the disease has been more or less destructive each year beginning



FIG. 1. Tomato plants with late-blight symptoms. (A, B, C' photographed at Jacksonville, Texas, on April 15, 1945. A, B: Plants from Lower Rio Grande Valley of Texas. (C, D: plants from cold frame.) A. Tomato stem that was typically twisted and broken by a late-blight canker. B. Tomato stem with a longitudinal crack in a late-blight canker; also a cross section through the hollow canker. C. *Phytophthora infestans* killed the leaves, grew into the stem, and caused light brown cankers in cold-frame plants. D. New growth on a tomato stem, the top of which had been killed earlier by late blight; photographed on May 25, 1945.

² Altstatt, G. E. Late blight of potatoes and tomatoes in the lower Rio Grande Valley of Texas. U. S. Dept. Agr. Plant Dis. Rptr. 29: 233-234. 1945.

with an epiphytotic³ in 1931. Earlier descriptions of tomato late blight were by Kern and Orton⁴ and by Giddings and Berg.⁵

The largest loss from late blight occurred on a farm near Jacksonville where nearly 17 acres were set with young tomato plants from the Lower Valley. On April 15 most of the plants were dead. Typical symptoms of late blight and spores of the causal fungus were found on the remaining plants. Plants dying early had been reset 3 times with locally grown plants, and they also had died from late blight. Late blight in the stems caused prominent cracking, shriveling, twisting, and hollowness of the cankered areas (Fig. 1, A, B). Many of the tomato tops were broken over at the canker. The *Phytophthora* spores evidently had been carried by wind from the blighted plants in the field to tomato seedlings in a nearby cold frame. By April 15, late blight had injured or killed the tops of more than 10,000 plants in this cold frame, giving the seedlings a brown, scorched appearance (Fig. 1, C). When the cold frame was examined again on May 25, some of the plants were still alive and new tops had developed from branches below the cankers after the spread of the late-blight pathogen had been arrested by warm, dry weather (Fig. 1, D). On another farm, a grower had "heeled-in" Lower-Valley tomato plants beside a hot bed. All of the seedlings in the hot bed and nearly all of the Lower-Valley plants that were set in the field were killed by the late-blight fungus.

These observations indicate that *Phytophthora infestans* may be transmitted from one region to another through movement of infected tomato plants, and under favorable weather conditions an epiphytotic may occur in the newly infested area.—P. A. YOUNG, Tomato Disease Laboratory, Texas Agricultural Experiment Station, Jacksonville, Texas.

The Eradicant Action of a Fungicide on Colletotrichum lilii in Lily Bulbs.—Preliminary studies by Plakidas¹ on the control of black scale of the Easter Lily (*Lilium longiflorum* Thumb. var. *eximium*) caused by *Colletotrichum lilii* Plak. by treating diseased bulbs with fungicidal dips or dusts indicated that mercuric chloride, borax, basic copper sulphate, calcium hypochlorite, and brilliant green were ineffective. In most cases the treatments also caused considerable injury. Continuing this work, tests have been made with several of the new organic fungicides. The results obtained with one of these materials were of sufficient promise to justify a preliminary report.

A method of rapidly evaluating the effectiveness of the fungicides in the laboratory was devised. Scales bearing lesions were removed from diseased bulbs and treated, after which they were placed in sterile water for 24 hours.

³ Taubenhaus, J. J., and W. N. Ezekiel. Late blight of potatoes and tomatoes. Texas Agr. Exp. Stat. Circ. 60. 1931.

⁴ Kern, F. D., and C. R. Orton. *Phytophthora infestans* on tomato. *Phytopath.* 6: 284-287. 1916.

⁵ Giddings, N. J., and Anthony Berg. A comparison of the late blights of tomato and potato. *Phytopath.* 9: 209-210. 1919.

¹ Plakidas, A. G. Black scale: A disease of Easter Lily bulbs. *Phytopath.* 34: 556-571. 1944.

They were then soaked for 4 to 5 minutes in a saturated solution of calcium hypochlorite for surface sterilization and plated on potato-dextrose agar. In check tests with scales immersed in tap water for 24-48 hours, fungus growth occurred from all scales, with *Colletotrichum lilii* constituting 50 to 75 per cent of the fungi present (Fig. 1). By using this method, a number of organic materials were eliminated as ineffective. Two materials, how-

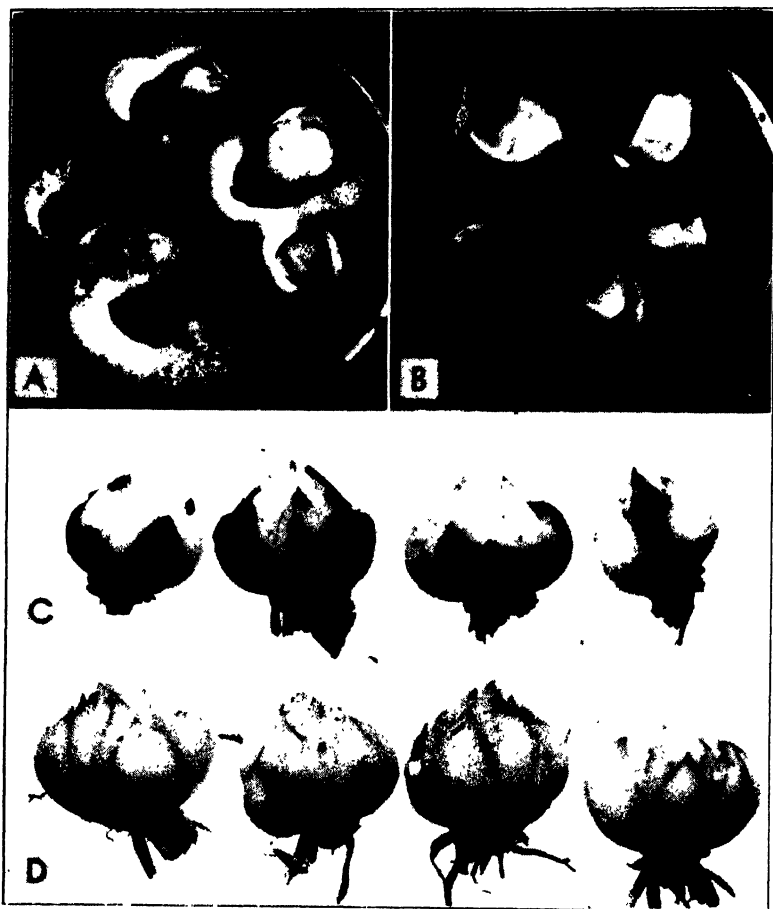


FIG. 1. Effect of Puratized N5X and N5E on the survival of *Colletotrichum lilii* in lily scales and bulbs. A. Nontreated scales on potato-dextrose agar, showing fungus growth. B. Scales treated with Puratized N5E, 1-2000, for 48 hours, on potato-dextrose agar, no fungus growth. C. Nontreated infected lily bulbs after 7½ months' growth in greenhouse. D. Lily bulbs treated with Puratized N5X, 1-2000, for 48 hours and grown in greenhouse for 7½ months.

ever, Puratized N5X and its improved form N5E (10 per cent phenyl mercuri triethanol ammonium lactate) at concentrations of 1-500 to 1-4000 where the treatment time was 24-48 hours, proved effective in killing the fungus in the scales. Reducing the treatment time to six hours failed to destroy the fungus even at concentrations of 1-250, indicating that the duration of the treatment was more important than the concentration of the fungicide.

These tests with Puratized N5X in the laboratory were followed by greenhouse tests with diseased whole bulbs. Representative results from these tests are given in table 1. Nontreated and treated bulbs from this test are shown in figure 1, C and D.

As in the laboratory experiments the effectiveness of the treatment was very much reduced when the treatment time was less than 24 hours regardless of concentration. In no case was injury to the bulbs or plants observed except where the treatment was for 24 hours or more at concentrations of 1-500 or greater. While emergence was reduced at these high concentrations, growth of the plants which emerged was not apparently affected.

In a field experiment in the 1944-45 season five lots, totaling 8 bushels, of heavily infected bulbs were treated with Puratized N5X, using different combinations of time of treatment and concentration of the fungicide. Be-

TABLE 1.—*The effect of treatment with Puratized N5X on the development of black scale on infected lily bulbs in pot experiments. Bulbs examined 7½ months after planting.*

Treatment	Concentration	Time	No. bulbs clean	No. diseased bulbs in classes ^a			Disease index ^b
				1	2	3	
Puratized N5X	1-1000	24 hr.	19	1	0	0	1.7
Do	do	48 hr.	15	1	0	0	2.1
Do	1-2000	24 hr.	10	0	0	0	0.0
Do	do	48 hr.	21	1	0	0	1.5
Do	1-1000	6 hr.	12	2	1	4	28.7
Do	1-500	do	16	2	3	2	20.2
Check			3	4	2	9	64.8

^a 1 = mildly diseased; 2 = moderately diseased; 3 = severely diseased.

^b Clean, mild, moderately, and severely diseased bulbs were given numerical values of 0, 33.3, 66.6, and 100, respectively. The disease index for any given treatment was obtained by summing the products of the numbers of bulbs in each class and the numerical values of the classes and dividing by the total number of bulbs in the treatment.

cause the planting was in a quarantine area, it was not feasible to include an untreated control. However, the trend of disease development in lots receiving the different treatments corroborated the laboratory and greenhouse results. At concentrations of 1-1000 and 1-2000 of Puratized N5X for 48 hours the disease indices were 3.1 and 4.5, and the actual percentages of diseased bulbs were 5.7 and 8.7, respectively. At a concentration of 1-4000 for 24 hours, 28 per cent of the bulbs were diseased and the disease index was 15.2. In the field as in the greenhouse there was no evidence of injury.

In September and October, 1945, extensive plantings were made with bulbs treated with Puratized N5E under a wide range of conditions. While most of the bulbs were treated for 48 hours with a 1-2000 solution, in more limited treatments the concentration and the length of treatment were varied. Approximately 2,000 bushels of bulbs were treated, and by January, 1946, satisfactory stands were present in all cases. This indicated that this material may be used in the field with safety.—F. J. LeBEAU, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana.

ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT
THE THIRTY-SEVENTH ANNUAL MEETING OF
THE SOCIETY, ST. LOUIS, MISSOURI,
MARCH 27 TO 30, 1946

Fungi Causing Diseases of Sugar-beet Seedlings in Montana. AFANASIEV, M. M. Numerous isolations were made from diseased sugar-beet seedlings grown in the field and in the greenhouse. Isolates include representatives of the following genera: *Phoma*, *Fusarium*, *Macrosporium*, *Rhizoctonia*, *Pythium*, and *Aphanomyces*. Beet seedlings in either chloropicrin-disinfected or steam-sterilized soil in the greenhouse were inoculated with many of these organisms. *Fusarium*, *Macrosporium*, and *Rhizoctonia* were only slightly pathogenic, *Phoma* and *Pythium* were moderately so, and *Aphanomyces* was the most pathogenic on sugar-beet seedlings. The symptoms produced by *Aphanomyces* on sugar beets grown in the greenhouse resemble those of the diseased seedlings in the field. Seedling diseases of sugar beets, from the standpoint of the organisms involved, are complex, and it is believed that in Montana *Aphanomyces* is more important as a pathogen than is any of the other fungi mentioned. Some of the fungi studied are only weak parasites and possibly act as secondary invaders.

A Bean Virosis of Importance in Montana. AFANASIEV, M. M., and H. E. MORRIS. For a number of years a virosis was observed on Great Northern bean, a variety resistant to bean virus 1. This virus closely resembles bean virus 2. Bean leaves are mottled, distorted, and crinkled, and older plants become rugose, dwarf, and spindly. Plants grow slowly, bloom late, and yield poorly. The virus is not transmitted through the seed. Infection resulted when the infectious plant juices were inoculated into Great Northern beans U.I. 15, U.I. 59, Mont. 1; Red Kidney; Pinto; Ideal Market; and on white and yellow sweet clover. Results were negative when red, Alsike, Ladino, and Dutch White clovers and alfalfa were inoculated. Although symptoms varied, all bean varieties were equally susceptible except for Great Northern Montana 1 which was somewhat less susceptible. Inoculated leaves of white and yellow sweet clovers had a chlorotic mottling. Bean varieties became infected when inoculated with juice from field-grown white and yellow sweet clovers and Red Kidney beans having symptoms similar to inoculated plants grown in the greenhouse. The sweet clovers or some other leguminous plants probably may be the source of infection on beans. The virus was inactivated in a dilution greater than 1:100; by heating for 10 minutes at 60° C.; and by aging in vitro for 18 hours or longer.

Brown Stem Rot of Soybean Caused by an Unidentified Fungus. ALLINGTON, WILIAM B. This disease appeared in a few fields in Central Illinois in the fall of 1944, and in 1945 it occurred in severe epidemic form in Central Indiana, Illinois, and Iowa. The damage in Central Illinois was extensive, reaching 10 per cent in some counties. Complete loss of yield occurred in some fields. Symptoms consist of browning of the pith and xylem of the stem, starting at or below the soil level and progressing slowly upward with only slight external symptoms in evidence in the form of occasional blighting of lower leaves. Then with unusual rapidity the leaves in the tops of the plants develop an interveinal chlorosis that is quickly followed by necrosis. In advanced stages the outside of the stem appears brown and the plants lodge badly because of extensive internal rotting. Temperatures must be low for disease development. The fungus appears to be soil-borne and generally present in the Midwest. The dense, putty-colored, nonfruiting mycelium grows slowly on all media tested. Isolation and artificial inoculation are readily made by standard methods. A few fields have indicated that continuous cropping to soybeans may increase the disease damage.

Effect of Growth Substances on Glomerella cingulata. ANDER, J. O. Fifteen growth-promoting substances were tested on 4 monospore lines of the organism by addition to a mineral-salts-glucose medium containing purified agar. Colonies were grown comparatively in Petri plates. Growth, as measured by colony diameter, increased in all lines when choline chloride was added. One line responded to the addition of niacin and another to the addition of thiamine.

A Chromogenic Line of Glomerella cingulata Derived from an Ascospore. ANDER, J. O. Six ascospores isolated from a single ascus produced 5 typical plus colonies and 1 red colony. The red culture produced conidia abundantly but never formed perithecia, either alone or when mated with plus or minus lines. It appears to be identical with another chromogenic line that was isolated from an apple by M. C. Goldsworthy.

Manganese Toxicity, a Factor in the Cause of Internal Bark Necrosis (Apple Measles). BERG, ANTHONY, and GENEVIEVE CLULO. Investigations for 6 years showed that the tissues of apple trees affected with this disease usually contained abnormal amounts of manganese. Accordingly, the rôle of manganese was determined in a series of greenhouse experiments. When Red Delicious trees were grown in soil which had been taken from orchards where the disease was severe and to which manganese was added both the number of plants affected and the severity of the disease increased. Rome Beauty, a variety normally not subject to the disease, when grown in similar soil and similarly treated did not become affected, although the tissues took up large amounts of manganese. Red Delicious trees grown in soil obtained from locations where the disease did not occur became diseased when manganese was added. The controls in this soil did not contract the disease. Red Delicious trees grown in sand culture to which varying amounts of manganese were added also became diseased at the higher concentrations of manganese. In all cases the disease appeared in late summer or early fall and developed with great rapidity after the first signs became visible. Preliminary experiments indicate that incorporation of lime in the soil inhibits absorption of manganese and tends to prevent this disease.

Diseases of Teosinte in Mexico. BORLAUG, N. E. Two smuts have been collected on teosinte (*Euchlaena mexicana* S.) in the vicinity of Amecameca, Mexico. One was identified as *Ustilago Kellermanii* C., while the second has been tentatively classified as *Sorosporium*. Both staminate and pistillate inflorescences and their supporting tissues are affected by *Sorosporium*, this smut being distinctly different from *U. Kellermanii*. Although a large number of collections of teosinte have been made in various parts of Mexico during the past year, smuts have been found only in the one area. By far the most common disease of teosinte throughout its range in Mexico is leaf rust (*Puccinia sorghi*) which has been collected from widely separated geographic areas.

***Puccinia sorghi* on Corn in Mexico.** BORLAUG, N. E. Recent studies have demonstrated the existence of corn rust throughout Mexico. However, for many years there has been considerable selection for rust resistance on a regional basis. In 1944-1945 collections of lines and varieties of corn from Mexico, the United States, Cuba, Venezuela, Hawaii, and Guatemala were grown in field tests at Chapingo, Mexico. Tremendous differences were observed in their reactions to races of *Puccinia sorghi* in that area. Reaction varied from a high degree of resistance to complete susceptibility and early death. In general, varieties native to the area in which tests were made were resistant; lowland corns were highly susceptible and many hybrids, inbreds, and varieties from other countries were completely susceptible. The distribution of races of *Puccinia sorghi* in Mexico is being studied along with the significance of the 3 species of *Oralis* which rust heavily each year.

***Tubercularia Canker and Dieback of Ulmus pumila* L.** CARTER, J. C. In August, 1939, branch and trunk cankers were observed on several trees 4 to 10 feet tall in a commercial nursery planting of Siberian elm (*Ulmus pumila* L.). Single-spore isolations were made from black sporodochia that had developed in the cankerous bark. The fungus isolated was a species of *Tubercularia*, apparently undescribed. Seven trunk and 18 branch inoculations were made on 21 Siberian elms on April 6, 1940. Infection developed from 6 of the trunk inoculations and 17 of the branch inoculations, and subsequently cankers were produced. In general, infection became evident within 10 days and sporodochia became visible in about 20 days. Cankers on trunks and branches continued to enlarge until mid-June, at which time callus tissues began to develop at their margins. By late August many of the cankers were covered with callus. Cankers produced by both inoculations and natural infections failed to enlarge in succeeding years. Branches which had been inoculated gradually died downward from the point of inoculation, usually to distances of 4 to 8 inches by mid-June.

A New Race of *Cercospora oryzae* on Rice. CHULTON, ST. JOHN P., and E. C. TULLIS. Previous to 1944 the rice variety Rexoro was resistant to the various races of *Cercospora oryzae* known to occur in Louisiana and Texas. In 1944, this variety was found with susceptible-type lesions. In 1945, the disease had spread over nearly all the Louisiana and Texas rice-growing areas. In inoculation tests, the varieties Blue Rose, Blue Rose 41, Caloro, and Fortuna, which are used to separate the 4 known races of *C. oryzae*, were resistant to cultures of this new race from Louisiana and Texas. Texas Patna and Blue-bonnet, two new varieties recently released, for which Rexoro was one of the parents, were both susceptible to the new race. All F_2 generations in crosses between resistant varieties and Rexoro segregated in a 3:1 ratio, with 3 resistant and 1 susceptible. (Louisiana Agricultural Experiment Station and U. S. Department of Agriculture.)

Genetic Variation in Gibberella zeae in Relation to Adaptation. CHRISTENSEN, J. J. As a result of genetic variation a monoconidial isolate of *Gibberella zeae* appeared to develop increased tolerance to malachite green, mercuric chloride, and ethyl mercury phosphate. When grown on potato-dextrose agar containing various concentrations of these toxic substances the fungus produced many distinct variants differing from their parent in cultural characters, physiology, pathogenicity, and morphology. On malachite-green agar some variants grew more than 100 times faster than their parent, while others grew at slower rates. These differences have persisted for many cultural generations. In the absence of mutation, there was no correlation between the number of generations a line had grown on a toxic medium and its ability to grow on it. However, the source of inoculum had a decided effect on the ability of certain variants to begin growth on a toxic medium. Variant 1-2 failed to grow readily on malachite green agar when the transfer was made from Smith-Humfeld agar but grew perfectly well when taken from potato-dextrose agar. A mixture of several distinct variants was grown for several cultural generations on potato-dextrose agar; then, when grown on toxic media, the component lines assorted themselves so that they could readily be recognized.

The Effect of Shading Techniques on Transmission of Tobacco-mosaic Virus through Dodder. COCHRAN, G. W. In transmission experiments, virus-free dodder, *Cuscuta campestris*, established on healthy potted tomato plants was passed through paper barriers and connected to tomato plants infected with tobacco-mosaic virus. In one experiment, 75 per cent transmission occurred when healthy plants were starved by holding in darkness, with all growing points removed from the connecting dodder. No transmission of virus occurred to an equal number of unstarved healthy plants connected by unpruned dodder. In another experiment, all dodder growing points were removed after connections were established. All diseased plants and half of the healthy plants were held under continuous light. The remainder of the healthy plants were starved of carbohydrates by bagging. Dodder connections were maintained for 2 weeks. Virus was transmitted to 83 per cent of the carbohydrate-starved plants and to only 3 per cent of the unstarved plants. The removal of all dodder growing points resulted in a channeling of carbohydrates and virus directly across the dodder stem from plant to plant. The direction of carbohydrate movement through this dodder channel was apparently determined by the carbohydrate gradient. A high carbohydrate level in diseased plants and a very low level in healthy plants favored virus transmission.

Ring Spot, A Common Contaminant of Stone Fruit-Virus Cultures. COCHRAN, L. C. In host range studies with stone fruit viruses, if we infect a suspect host of a different species with inoculum from a mother plant and, in turn, reproduce similar symptoms on the mother plant species by transfer from the infected suspect host, we conclude the symptoms produced in the suspect host to represent those of the virus causing the disease in the mother plant. This technique is not sufficient to show that the mother plant was not carrying two viruses, one producing symptoms in the original or mother plant and existing latent or obscured in the second or suspect host, whereas the other existing latent in the mother plant is responsible for symptoms in the suspect host, both of which were transmitted in each transfer. The ring-spot virus has been found widely existent in both cultivated and wild prunus species in nature. This virus is a complex with many forms, most of which produce symptoms only in the early stages of infection and some of which infect certain hosts without symptoms. Source material of other viruses and commercial nursery stock has commonly been found carrying the ring-spot virus without visual symptoms. Conclusions drawn from work with contaminated stock will result in confusion in the literature.

Interaction of Some Forms of the Peach-mosaic Virus. COCHRAN, L. C., and JOHN L. RUE. Extended studies have demonstrated the existence of a multiplicity of forms of the peach mosaic virus capable of producing a symptom gradient on Hale peach ranging from a severe effect to one so mild that diagnosis is doubtful. Early work indicated that forms producing mild effects commonly arise in trees affected with severe forms and can be obtained from mildly affected sport-like branches. If trees developed from these sport-like branches were reinoculated from the "severe" portion of the mother tree, no increase in severity of symptoms resulted. Bodino found that mild forms protect against severe forms from the same locality in Colorado. In many combinations the authors have obtained conflicting results. A mild form arising in Texas inhibited symptom production by a severe form from Texas but failed to interfere with a severe form from Arizona. Similarly an Arizona mild form protected against the Arizona severe form but failed to inhibit the Texas severe form. In other combinations mild forms variously moderated the symptoms of severe forms as compared with checks. It thus appears that the peach-mosaic virus is a complex of variously related forms which compete in peach.

A Wilt and Root Rot of Asparagus officinalis L. var. altilis L. COHEN, SYLVAN IRVING. *Fusarium oxysporum f. asparagi* causes a root rot and vascular wilt in asparagus in the United States which is distinct from a foot rot in Germany caused by *F. culmorum*. It may affect 25 to 50 per cent of a planting, producing yellowing, stunting, and wilting of the growing stalks. Vascular discoloration occurs within affected stems, roots, and crowns, and it is associated with reddish brown lesions and rotting. When nurseries are established in contaminated soils, a severe damping-off occurs in circular areas. Single-spore isolates of the fungus from Washington, California, and South Carolina proved to be morphologically and culturally indistinguishable and were equally pathogenic. A soil temperature of 28° C. and sandy soils favor the development of the disease. Because of the anatomy of asparagus, severe symptoms may appear one year and not the next, although the plant is still infected. No effective control measures are known. Observations of field plots reveal that fertilizers have no significant effect upon the disease. Twelve species and varieties of the genus *Asparagus* proved equally susceptible. What appears to be the same disease has been reported since 1908 as "stem rot," "dwarf asparagus," "stem canker," "root rot," and "wilt."

Plant Pathology in the U. S. Navy in Wartime. COOK, HAROLD T. Duties performed by the author while an officer in the U. S. Navy in the South and Central Pacific areas in World War II demonstrated that the training and experience of plant pathologists may be applied profitably to problems of production, procurement, and handling of food supplies for the Armed Forces and indicated that plant pathologists may be used efficiently in the Armed Forces in future wars. Duty in the South Pacific was to organize and direct inspection of fresh, canned, dehydrated, and quick-frozen fruits and vegetables obtained from New Zealand. This involved preparation of specifications, training and supervision of inspectors, and establishment of procedures and policies. Additional duties were to increase production and develop improved methods of harvesting, packing, and storing. Surveys were made of the availability of certain food supplies. A manual for cool storage and handling of fresh vegetables was prepared. Refrigerated shipments to the Forward Areas were studied and improved methods of loading and handling such cargoes were instituted. Assignment in the Central Pacific was to command a Naval Unit engaged in growing fresh vegetables on the islands. Production in those areas involved numerous cultural, disease, and insect problems.

Helminthosporium Foot Rot of Barley. DICKSON, J. G. In 1943 and 1944, barley yields were low throughout the North Central spring-barley area, where Wisconsin Barless occupies most of the acreage. Late sowing and conditions favorable for attack by *Helminthosporium sativum* Pam., King, and Bakke were among the factors that apparently accounted for this barley failure. Temperature and moisture experiments in the greenhouse have demonstrated the importance of *H. sativum* foot rot and the relative susceptibility of Wisconsin Barless in contrast to that of Oderbrucker. The inoculum used was a composite of *H. sativum* cultures isolated from barley kernels obtained through the area in the 2 years. Clean seed of both varieties was inoculated with a conidial suspension and sown in soil, after which the fungus growing on oat hulls was incorporated into the surface layer of the soil. The plants were grown in the greenhouse to the fully headed stage at 52° and at 70° F. in moderately dry and wet soils. Moisture was maintained by subirrigation. In both varieties, less seedling blight occurred at the lower temperature and in the drier soil than in the wet soil at the higher temperature. At 70° F, foot rot was severe in both the moderately dry and wet soil, Oderbrucker having 60 per cent healthy plants in contrast to 15 per cent in Wisconsin Barless. At 52°, Oderbrucker had 91 per cent healthy plants and Wisconsin Barless 81 per cent. (Wisconsin Agricultural Experiment Station and U. S. Department of Agriculture.)

Spread and Control of Oak Wilt. DIETZ, S. M. and JAMES W. BARRETT. Oak wilt has been found in Illinois, Iowa, Minnesota, Missouri, and Wisconsin. This disease, caused by *Chalara quercina* Henry and first recorded in Iowa in 1932, occurred throughout the State in 1944 and 1945, being most severe in the eastern part. It has been epiphytotic on *Quercus alba*, *Q. borealis maxima*, *Q. ellipsoidalis*, *Q. imbricaria*, *Q. macrocarpa*, and *Q. velutina*. Using Koch's postulates, the 13 species of oaks recorded as native to Iowa have been proven as hosts for *Chalara quercina*. The rate of spread, which occurred in concentric zones from the initial point of infection, varied with the season and the species of oak. Initial infection is in the crown of the tree and the fungus progressively infects leaves, stem, and roots. The fungus spreads more rapidly in red oak trees than in white or burr oak. The symptoms varied with the species of oak. The fungus rarely overwinters on red oak, usually on white and burr oak. It has been isolated repeatedly from dead twigs 6 months after infected trees were felled. For 2 years various sanitation methods have checked and sometimes controlled the spread of oak wilt.

Improvement of Ascorbic Acid Content in Yellows-resistant Cabbage. FOSTER, R. E., and J. C. WALKER. Individual plants of several standard or improved varieties of cabbage homozygous for the gene for type A resistance to yellows were crossed with individuals from a Wisconsin All Seasons line high in ascorbic acid content but containing only type B resistance to yellows. F_1 and F_2 progenies were studied to obtain individuals with high vitamin C content, desirable varietal type, and type A resistance to yellows. Ascorbic acid content was controlled by multiple factors. Breeding lines of cabbage varied in the number of factors controlling this character. Transgressive segregation toward increased vitamin C content was apparent in some of the F_2 progenies. By use of the individuals selected, there may be developed lines of several commercial varieties having a greatly increased ascorbic acid content as well as homozygous type A resistance to yellows.

Differences in Diploid Lines of Ustilago zeae. GATTANI, MOHAN LAL. Several diploid lines appeared in the progeny of cross 410, between the relatively stable haploid line 10A, and the mutable haploid line 17D₁. Seven diploid lines differed from the parents and from each other in the following characters: 1) appearance on nutrient media; 2) rate of growth and enzyme production; 3) adaptability to arsenic and malachite green; 4) ability to cause anthocyanin production on 4 selfed lines of corn; 5) pathogenicity on corn plants. One of the diploid lines, 410qq, intermediate between the parents in most cultural and physiologic characters, was mutable like the 17D₁ parent. Numerous mutations were induced by growing 410qq on media containing lithium, arsenic, or uranium. Some of these mutants closely resembled the mutable haploid parental line 17D₁ or its haploid mutants; moreover, some of the mutants of the diploid 410qq were haploid, as indicated by their inability to infect corn plants alone. When these mutants were paired with 10A₁, however, normal infection resulted, thus indicating that dissociation of diploid line 410qq resulted in the production of haploid lines similar in many respects to 17D₁, one of the original parents.

An Actinomyceete Inducing Wood Necrosis and Gummosis in Citrus. GODFREY, G. H. An unidentified Actinomyces-like organism produces a distinctive type of plant disease--a wood necrosis and gummosis of citrus trees--which is the most serious citrus tree disease in the Lower Rio Grande Valley of Texas. The symptom is a meandering band of necrotic wood, well beneath the bark. It is light ochraceous buff to ochraceous buff (Ridgway), bordered in the region of advancing infection by a narrow band that is apricot orange to rufous. Where an advancing band grows outward to the cambium, that tissue is killed, the bark cracks, and gum exudation varies from slight to extreme. This is the prominent external symptom of the disease. Severely affected branches are killed, and after infection has spread well into the wood in the trunk, the entire tree dies. Histological studies show the organism, with very small but clearly defined hyphae and spores with dimensions within the range of bacteria, to be abundant in affected tissues. It clogs the xylem tubes and invades the cells in the medullary rays. Inoculations into healthy branches have consistently reproduced the disease. Progress has been made on control by (1) pruning off badly affected branches, (2) excavating affected wood and treating the exposed healthy wood with a penetrating disinfectant, (3) preventing new infections by avoiding wounding of the bark and by treating all pruning wounds.

Control of Decay in Citrus Fruits. GODFREY, G. H., and A. L. RYALL. In a six-year series of tests for Diplodia stem-end-rot control, chiefly on lemons in the Lower Rio Grande Valley, thirty materials were used. Most of them were unsatisfactory. Sodium ethyl mercuthiosulphate (Merthiolate) throughout the series consistently gave nearly perfect control, and it was used throughout as a standard for comparison with other materials. In 21 tests in concentrations of 1 to 10 and 15 thousand it averaged 1.2 per cent decayed fruits with many of the tests giving perfect control, when checks averaged 27 per cent stem-end decay and ranged from 5.5 to 86 per cent. Phenylmercuri 2-2-2 nitrilotriethanol lactate (Puratized N5E) and Thiourea also gave good control. Sodium orthophenylphenate was less efficient and required thorough rinsing after treatment to avoid serious chemical injury. Saturated borax solution and 5 per cent sodium metaborate both gave fair control but had a tendency to cause dull fruit and sometimes early wilting and shrivelling. The treatments that were most effective in the control of Diplodia stem-end rot usually also reduced the blue and green molds (*Penicillium spp.*); but in occasional lots in mid-winter such decay was high in spite of chemical treatments. The use of diphenyl-impregnated tissue as fruit wraps reduced blue mold to a point well below that occurring after fruit-dip treatments alone. In a typical case following sodium metaborate dip, there was 46.4 per cent blue mold in plain wraps and 6.7 per cent in diphenyl wraps; following Merthiolate there was 29.4 per cent in plain and 7.8 per cent in diphenyl wraps; in the checks there was 56.7 per cent in plain and 10.7 per cent in diphenyl.

Species of Fusarium Isolated from Samples of Cereal Seed in Canada. GORDON, W. L. A total of 18 species, varieties, or forms of *Fusarium* representing 9 sections of the genus, were isolated and identified from 1579 surface-sterilized seed samples of wheat, 1152 of oats, and 1042 of barley produced in the 8 seed-inspection districts in Canada during 1939 through 1943. *Fusarium poae*, *F. sporotrichioides*, *F. avenaceum*, *F. equiseti*, *F. scirpi*, *F. scirpi* var. *acuminatum*, *F. culmorum*, *F. graminearum*, *F. sambucinum*, and *F. oxysporum* were isolated from samples of wheat, oats, and barley; *F. scirpi* var. *compactum* and *F. moniliforme* from wheat and oats; *F. sambucinum* form 1 and *F. solani* from oats and barley; *F. arthrosporioides* from wheat; *F. lateritium* from oats; *F. semitectum* var. *majus* and *F. equiseti* var. *bulatum* from barley. *Fusaria* occurred in a greater percentage of the samples of oats and barley than of wheat; and in a greater percentage of the samples obtained from Eastern than from Western districts. Only a small percentage of the seeds of the 3 crops harbored *Fusaria*, the highest proportion in any of the districts being 2.6 per cent of the seeds of wheat, 8.5 per cent of oats, and 7.1 per cent of barley.

Control of Fusarium Wilt of Tomato with Dithane. GOTTLIER, DAVID, and J. W. NEUBERGER. Preliminary experiments with soils in pots in the greenhouse were made on the use of Dithane (disodium ethylene bisdithiocarbamate) to control the *Fusarium* wilt of tomatoes. Dithane was mixed with infested and noninfested sassafras loam soil at a rate approximating 100 lbs. per acre. The average emergence of tomato seedlings was 24.4 per cent greater in the treated soil than in the checks. In the same experiments, 95.3 per cent of the plants in nontreated infested soils wilted, but only 19.3 per cent wilted in those infested soils which contained Dithane. When seedlings were transplanted 3 months later to these experimental soils, 88.3 per cent of the plants wilted in the nontreated, infested soil and less than 1.0 per cent in the Dithane-treated, infested soil. This indicates that the fungicidal effect of the Dithane may last for some time with normal greenhouse watering. In noninfested soils the fungicidal treatment did not prevent or reduce the germination of tomato seeds, but seedlings were stunted when grown in treated soils.

Polymodal Dosage-Response Curve between Calcium-potassium Ratio and Potato Scab. GRIES, GEORGE A., JAMES G. HORSFALL, and H. G. M. JACOBSON. The literature on ecology of potato scab records disagreement as to relative importance of hydrogen, calcium, and potassium ions. Experiments have been made on 2 soil types in 2 years with calcium as sulphate and carbonate, and hydroxide and potassium as sulphate and chloride. The incidence of scab was measured by grading the tubers on the basis of percentage of area scabbed. The disease bore the expected relation to hydrogen ions, but when pH was constant, the curve for percentage of scab showed several modes of high scab separated by "valleys" of low scab as the applied Ca-K ratio increased in 3-fold units from 0.07 to 486. In that range there appeared to be about 3 peaks spaced about 9-fold apart. The curves matched well in both years if the experimental variables were held constant. Alteration of soil type or the cation, however, altered the position of the peaks and valleys. The basis for the polymodal curve seems to be that calcium and potassium act antagonistically in the production of potato scab. The degree to which they antagonize each other apparently depends on the relative amounts of each. The occurrence of polymodal curves is not uncommon in biological research where 2 antagonistic factors such as H and OH are varied simultaneously. The 2 factors need not be necessarily antagonistic so long as their effects do not alter at equal rates with dosage. (Purdue University Agricultural Experiment Station and Connecticut Agricultural Experiment Station.)

Burn-Blight of Jack and Red Pine Following Spittle Insect Injury. GRUENHAGEN, R. H., A. J. RIKER, and C. AUDREY RICHARDS. A serious disease, first brought to attention in 1941, has been killing jack and red pine progressively from top to bottom. In northeastern Wisconsin the damage now extends over 6000 acres on 64 areas in 9 counties. Evidence, which included completing Koch's postulates with 6 single-spore isolates, showed *Chilonectria cucurbitula* (Curr.) Sacc. to be the causal agent. The disease usually followed injury by the Saratoga spittle insect (*Aphrophora saratogensis* Fitch), which carried the fungus and introduced it while making numerous feeding punctures in the twigs. This insect was active from early July through September. However, the fungus usually appeared to incubate over fall and winter and induced most rapid necrosis from April to mid-July. Although the fungus usually entered through spittle insect-feeding punctures, it was found developing from wounds caused by other agencies. Jack pine on a poor site, over a 14-month period, had over 100 times more disease than comparable trees on a good site. Severe top and branch pruning did not increase the incidence of disease on jack pine. The most promising immediate preventive seems to be control of the insect vector. (U. S. Department of Agriculture, Wisconsin Conservation Department, and Wisconsin Agricultural Experiment Station.)

A New Race of Ustilago avenae. HANSING, E. D., E. G. HEYNE, and T. R. STANTON. A collection of loose smut was made from Fultex oats in Kansas in 1944 and identified as a new race in 1945. It is characterized by the resistance of the smut testers, Black Mesdag, Large Hull-less, Red Rustproof, and Fulghum (0 to trace smut), and the susceptibility of Richland, Monarch Selection, Green Mountain, Jounette, Victoria, Canadian, and Monarch (49 to 95 per cent smut). Of new varieties of oats distributed during the last 7 years or now ready for distribution to growers, Bonda, Mindo, Neosho, and New Nortex were highly resistant, having no smutted panicles. Goldwin, Benton, Clinton, Marion, and Mission were moderately resistant (1 to 3 per cent smut), whereas, Osage, Ventura, and Forvie were intermediate in susceptibility (9 to 26 per cent smut). Boone, Vieland, Cedar, Tama, Florilee, Fultex, and Traveler, all Victoria-cross selections, were highly susceptible (74 to 96 per cent smut). Regarding other varieties tested, Markton, Brunker, Bond, and Navarro were highly resistant (0 to trace smut), whereas, Kanota, Fulton, Trojan, and Otoe were moderately resistant (1 to 6 per cent smut). Columbia was intermediate in susceptibility (22 per cent smut). Of hybrid oat selections in the Kansas Advanced Yield Test, the Cooperative Uniform Spring Sown Red Oat Test, and the Cooperative Uniform Oat Smut Nursery, 65 of 105 selections were resistant (0 to 1 per cent smut) to this race. (Kansas Agricultural Experiment Station and U. S. Department of Agriculture.)

Effect of Bunt on the Development of Seedling Blight and Foot Rot of Wheat. HANSON, E. W. Ceres and Ulka wheat plants from seed inoculated with a mixture of several races of *Tilletia foetida* and *T. caries* developed more seedling blight and foot rot than plants from seed not inoculated with bunt when grown in the field or in nonsterile field soil in the greenhouse at St. Paul, Minnesota, in 1945. All experiments were of split-plot design and included 8 replications. Seedling-blight infection ratings increased from an average of 12 per cent in noninoculated plots to 40 per cent in inoculated plots. Similarly, foot-rot infection ratings increased from 31 per cent in the checks to 72 per cent in the plots inoculated with bunt. The combined effect of bunt infection and seedling blight caused an average reduction in seedling vigor of 13 per cent, as indicated by green weight, and a reduction in seedling stand of 8 per cent, as compared with plots not inoculated with bunt. The average stand at maturity in plots having both bunt and foot rot was 16 per cent less than for plots having foot rot only. There was no significant effect of bunt on the average number of tillers per plant. (United States Department of Agriculture and Minnesota Agricultural Experiment Station.)

Effect of Fusarial Head Blight on the Development of Bunt of Wheat. HANSON, E. W. Fusarial head blight reduced the percentage of heads with bunt in 92 out of 110 hard red spring and durum wheats in preliminary experiments at St. Paul, Minnesota, in 1945. Duplicate series of the wheats were inoculated with a mixture of several races of *Tilletia foetida* and *T. caries* and sown in adjacent field plots. Both plots were treated identically until the wheats reached the heading stage when one plot was sprayed almost every evening with a spore suspension of *Gibberella zeae* and *Fusarium* spp. until an epidemic of head blight developed. The other plot was not sprayed. The reduction in smutted heads due to the presence of head blight varied from 0 to 48 per cent, depending on the variety and on other factors. (U. S. Department of Agriculture and Minnesota Agricultural Experiment Station.)

Recent Changes in the Stem-rust Situation in Mexico. HARRAR, J. G., W. Q. LOEGERING, and E. C. STAKMAN. For more than a decade the distribution of races of *Puccinia graminis tritici* in Mexico has differed in different regions. Population trends of races in Northeastern Mexico have paralleled fairly closely those in the United States. In the southern wheat-growing regions of Mexico, on the other hand, only races 38, 59, and 19 have occurred in appreciable amounts, thus indicating relatively little effective interexchange of inoculum between Southern and Northern Mexico. In 1945, however, races 56 and 17 were found with sufficient frequency to cause apprehension that they may be establishing themselves in Southern Mexico. Moreover, race 8 of *P. graminis avenae* was found in Mexico for the first time in 1945 and was present as far south as Mexico City, where it caused heavy infection on certain varieties that derived their stem-rust resistance from Richland oats. A number of unusual races of *P. graminis tritici* have been found occasionally. It had been suspected but not proved that native species of *Mahonia* might rust at high elevations and result in the production of such races. This suspicion was confirmed in 1945 when the unusual race 16 was isolated from heavily rusted *Mahonia* bushes growing in the mountains near Mexico City. (Rockefeller Foundation in Mexico, U. S. Department of Agriculture, and Minnesota Agricultural Experiment Station.)

Field and Greenhouse Studies on 16 Tobacco Hybrids and Varieties. HENDERSON, R. G. Seven commercial varieties of flue-cured tobacco and 9 lines resistant to root rot

(*Thielaviopsis basicola* and other organisms), selected from the progeny of crosses between commercial varieties and Turkish tobacco, were planted in 1/80-acre plots of soil only lightly infested with root-rot organisms. Plant height and leaf length and width were measured 5 times during the season. Leaves were harvested, cured, and graded in the usual manner. Plants of each of the 16 varieties were grown in pots in the greenhouse under conditions favorable for root-rot infection. When about 6 inches high, plants were removed from the pots; the roots were carefully washed, inspected, and scored according to the severity of infection. Six hybrids were highly resistant and 3 were intermediate. One commercial variety, Yellow Special, was moderately resistant but all the others were very susceptible. In the field, all of the resistant lines had wider leaves than the susceptible varieties, but leaf width was not directly proportioned to the degree of resistance. Since root rot was not a serious factor in the field test, the degree of resistance was not reflected in the yields.

Seed Treatment and Other Tests with Soybeans in Ontario. HILDEBRAND, A. A., and L. W. KOCH. In a 2-year series of field experiments involving plots planted by hand in a randomized, five-replicate design, the efficacy of Spergon (3 oz. per bu.), Arasan (2 oz.), and Fermate (2 oz.) was tested on seed lots of the variety, A. K. Harrow, which differed widely as to germinability, disease potentiality, and degree of seed-coat injury. With poor quality seed and the cracked-coat fraction of an otherwise high-quality seed, Spergon accelerated and increased emergence of seedlings and increased yield. In no other instance was increased emergence correlated with statistically significant increases in yield. While the beneficial effect of Spergon was consistent so far as seed of low quality or damaged seed were concerned, such was not the case in regard to seed of high quality. Spergon was consistently more effective than Arasan or Fermate, however, in reducing the incidence of disease or abnormality in early-season stands of plants. Results indicated a complete lack of correlation between yield and stand of plants, the stands differing numerically by a ratio almost as high as 4:1.

Wilting Induced in Tomato Cuttings by Toxic Substance from Crown gall Bacteria. HODGSON, ROLAND, W. H. PETERSON, and A. J. RIKER. Sterilized filtrates from fermented cultures of *Phytophthora tumefaciens* (Smith and Town.) Bergey *et al.* induced a wilting of tomato cuttings, which affected largely the leaflets, while the stems and petioles remained turgid. Under standardized conditions, the approximate quantity of toxic substance in filtrates was measured by an assay involving filtrate concentration, solution intake, and severity of wilting (toxic index). The substance causing the wilting was thermostable in neutral solution, nonvolatile, relatively insoluble in most organic solvents, and dialyzable. After alcoholic fractionation of the filtrate, much of the toxic activity was found in a concentrate consisting largely of a previously studied glucosan. Several tests indicated an association between toxic substance and the polysaccharide. Therefore, the purified glucosan (previously reported) was tested and found to account for a large part of the toxic activity. Inulin and soluble starch (0.4 per cent aqueous solution) also induced similar effects. An accumulation of polysaccharide was found in the leaves of cuttings wilted by the filtrates; this accumulation was greater in the wilted portions than in the nonwilted portions of the leaves.

The Physiology and Pathogenicity of Strains of Corticium solani. HIRSTON, BYRON R. A physiologic and pathogenic comparison was made of a number of isolates of *Corticium solani* (Prill. and Del.) Bourd and Galz. (*Rhizoctonia solani* Kühn) obtained from a wide range of hosts. The fungus required small quantities of zinc, iron, copper, and manganese for most rapid growth in a synthetic nutrient solution. No other micro element was found to influence the growth rate at a level below that of toxicity. Zinc and iron proved most important; however, neither zinc nor iron alone or combined individually with copper and manganese significantly increased the growth rate. Only when both zinc and iron were present did copper and manganese further accelerate the growth rate. Isolates varied in ability to utilize the various carbon and nitrogen sources employed. The hexose sugars were the most readily available forms of carbon. Additions of certain amino acids accelerated growth particularly during the early stages. Isolates differed in pathogenicity. Some were completely nonpathogenic on all hosts tested; some had a high degree of specialization, being pathogenic on only one host; and others were pathogenic on nearly all hosts.

The Mode of Vector Feeding and the Tissues Involved in the Transmission of Pierce's-disease Virus in Grape and Alfalfa. HIRSTON, BYRON R., KATHERINE ESAU, and WM. B. HEWITT. Feeding punctures of the following vectors of Pierce's-disease virus on grape and alfalfa were studied: adults and nymphs of *Draculacephala minerva* Ball and *Neokolla circellata* Baker; adults of *Heliochara della* Oman and *Carniocephala fulgida* Nott.

All vectors sought the xylem tissue in the process of feeding. The mouth parts reached xylem through the phloem or through the medullary ray. They passed between or directly through cells, including the tracheary elements. Using *D. minerva* on grape, 86.8 per cent of the 230 punctures studied reached the xylem, with 32.8 per cent of these passing through the phloem. On alfalfa 90.9 per cent of 451 punctures reached the xylem, and 15.6 per cent of these did not injure the phloem. The vectors transmitted the virus only when xylem tissue could be reached during the feeding process. Confinement of the 2 species of viruliferous vectors, *D. minerva* and *C. circellata*, to various portions of healthy grape and alfalfa plants resulted in the following percentage transmissions: whole plant, 82.1; whole stem, 80.0; exposed xylem strip, 65.1; phloem strip, 0.0. This appears to be the first record of insect transmission of a virus directly into xylem tissue.

Comparison of Several Fungicides as Dips for Seed Sweet Potatoes. JEFFERS, W. F., and C. E. COX. Various fungicides have been studied as prebedding dips for Maryland Golden seed sweet potatoes under field conditions. Several of the newer organic fungicides were superior to the generally recommended treatments. None of the newer materials controlled black rot better than did the standard treatments. However, they did not cause the delay in sprout production or decrease in the number of sprouts usually associated with dips of bichloride of mercury (1-1000), Semesan Bel (1 lb./7.5 gal.) and borax (1 lb./5 gal.). Outstanding during 2 seasons were Spergon (1 lb./4 gal.) and Phygon (0.5 lb./4 gal.). In 1945, Fermate (0.5 lb./4 gal.), Zerlate (0.5 lb./4 gal.), Thiosan (1 lb./4 gal.), Puratized N5E (1-4500) and Isothan Q15 (1-4500) also significantly increased the number of healthy sprouts produced.

Field Studies on the Mild Streak Disease of Black Raspberries. JEFFERS, W. F., and M. W. WOODS. During the past 10 years, mild streak has become the most serious disease of black raspberries in Maryland. Apparently virus in nature, its typical symptoms consist of dark-green or purplish, water-soaked streaks on new canes; curling of leaf tips; and dry, seedy fruits. For 4 years careful records have been maintained on the spread of mild streak in a planting originally streak-free. During this time the amount of the disease increased from 1.5 per cent in the first fruiting season to 46.7 per cent in 1945. Although infection at the end of the first season was rather uniformly distributed throughout the planting, there has been a much greater increase in disease incidence in the end of the field adjacent to an uncultivated area than in the end of the field surrounded by cultivated land.

A Virus Complex in Eryngium. JOHNSON, JAMES. Sea holly (*Eryngium aquaticum* L.) is an umbelliferous perennial species sometimes grown as an ornamental. The presence of naturally occurring mosaic-like symptoms suggested inoculation to tobacco for virus determination. At least 3 different viruses were present. One of these viruses, which at first confused efforts at isolation, was a mild or attenuated form of the common tobacco-mosaic virus. *Eryngium* is, however, not very susceptible to this virus or to non-attenuated strains; no distinctive symptoms are evident; the progress of the virus in the host is very slow and its concentration is often very low. The more common virus in *Eryngium* is apparently new, although it possesses many similarities, in properties and host range, to the viruses of the cucumber-mosaic group. Protective inoculation tests, however, do not suggest any relationship to ordinary cucumber mosaic, and differential hosts do not indicate relationship to the delphinium- or celery-mosaic viruses, which the new virus resembles in other respects. A third virus producing almost uniform chlorosis over the entire area of tobacco leaves is occasionally present in *Eryngium*.

Water-congestion and Infection Experiments on Various Plant Species. JOHNSON, JAMES. Natural water-congestion was induced in plants of 30 species after exposures of 1 to 24 hours in a moist chamber. Barley, corn, beans, tomatoes, tobacco, peas, and wheat were congested most readily, but differences between and within varieties were common. Alfalfa, clover, celery, and carrot were among 20 other species in which there was no macroscopically visible evidence of congestion. Outdoor-grown plants generally congested more easily and were more highly predisposed to infection with representative parasites than greenhouse-grown plants. With tobacco, 100 per cent of the outdoor-grown plants congested readily and were infected with the wildfire organism without wounding, while there was no infection on the greenhouse-grown controls exposed in the moist chamber at the same time. Efforts to reproduce the favorable "outdoor conditions" in the greenhouse during winter months have not been successful. Other nonwounded greenhouse-grown plant species are highly susceptible to infection by certain parasites (e.g., those of late blight of potato, anthracnose of bean, and rust of sunflower). With such diseases, it is as difficult to prove as to disprove the relation of congestive water to predisposition.

Normally resistant varieties of some species may often be predisposed to heavy infection by water congestion, provided other conditions are also favorable.

Control of Copper Spot on Fine Turf Grasses. KEIL, HARRY L. Copper spot (*Gloeocercospora sorghi*), as a new disease of fine turf grasses, was reported from Pennsylvania. It is now known to exist in many states, and it approached epiphytotic proportions on Piper Velvet Bent grass in Rhode Island during 1945. Spray treatments with 4 organic fungicides, at the rate of 10 gal. solution per 1000 sq. ft. of turf, were applied July 26 and 31. Treatments were replicated 8 times on blocks of 25 sq. ft. Average percentages of the area damaged by the disease were recorded September 10 and 27. Puraturf (phenyl mercuri triethanol ammonium lactate, 1:10,000), Puratized 177 (1:5000), and Zerlate (zinc dimethyl dithiocarbamate, 1.5 lb. per 100 gal.) gave a prolonged protection period of at least 41 days after treatment. Plots treated with Puratized 177 were still 99 per cent free from disease after 58 days, whereas disease had appeared on Puraturf and Zerlate plots. Up to 24 per cent of the grass was injured by disease on the nontreated plots, but plots treated with Phygon (2,3 dichloro 1,4 naphthoquinone, 1 lb. per 100 gal.) had 34 per cent disease. Standard treatments had failed to control copper spot in other experiments.

Inheritance of Pathogenicity and Sex Reaction in *Venturia inaequalis*. KEITH, G. W., CURT C. LEBEN, and J. R. SHAY. A monascospore line of *Venturia inaequalis* that incites typical scab lesions on Haralson and Wealthy and flecks on Yellow Transparent and McIntosh apple leaves was crossed with one that incites the converse disease reactions on these 4 varieties. The 8 ascospores were isolated in serial order from each of 35 progeny asci and the resulting lines tested for pathogenicity to the leaves of each of the 4 apple varieties and for sex reaction. In each ascus tested, segregation for pathogenicity to each variety as indicated by the lesion or fleck reaction was in 1:1 ratio. Of the 140 pairs of lines, 56 showed parental combinations for pathogenicity (inciting lesion or fleck on Haralson and Wealthy and the converse reaction on Yellow Transparent and McIntosh) and 84 showed new combinations (inciting lesions on all 4 varieties or flecks on all). There was no evidence of linkage between the alleles governing pathogenicity to Haralson and Wealthy and those governing pathogenicity to Yellow Transparent and McIntosh. The latter were shown to be linked with those governing sex reaction. The data on second division segregation indicate that the loci for pathogenicity dealt with herein are more than 33 cross-over units from their respective centromeres.

Corn Pericarp Injuries and Seedling Diseases. KOEHLER, BENJAMIN. Corn-seedling diseases under present seed-producing and seed-processing methods in the Corn Belt are caused chiefly by organisms residing in the soil, and the extent of damage is governed considerably by the extent of pericarp injury. A break directly over the plumule is the most injurious type. Breaks in the crown which expose the soft endosperm are second in importance. Breaks in the pericarp over the germ area to one side of or beyond the plumule, or directly over the radicle, are still less important. Of least importance are removal of the tip cap and breaks which expose only horny endosperm. In controlled inoculation experiments some strains of *Penicillium oxalicum* have been the most damaging to corn when the pericarp was injured, while some species of *Pythium* were second in importance. Isolation from naturally infected seedlings grown from injured seed in field soil seldom yielded *Penicillium oxalicum* but species of *Pythium* were commonly found. Considerable pericarp injury appears to be unavoidable under present processing methods. Fortunately, much benefit is obtained by treating with seed protectants. Arasan and Spergon have given somewhat better results on injured seed than the mercurials.

Viability of Stored Seeds of Forage Crops Treated with Different Fungicides. KREITLOW, K. W., and R. J. GARBER. Seeds of alfalfa, red clover, Ladino clover, and Sudan grass were treated with New Improved Ceresan, Semesan, Arasan, Spergon, and Yellow Cuproicide. Samples of the treated seeds along with nontreated controls were stored in closed and open containers at 10° and at 25° C. Germination was tested periodically in Petri dishes and in flats of soil. After 30 months, treated seeds of alfalfa, red clover, and Ladino clover stored at 25° showed no appreciable injury from any of the fungicides tested. There was no difference in germination between seeds stored in open and closed containers at 25°. Germination of Sudan grass treated with New Improved Ceresan was noticeably reduced 1 month following treatment and within 6 months none of the treated seeds germinated, regardless of storage temperature or condition. None of the other fungicides reduced germination of Sudan grass. At 10°, germination of treated and nontreated seeds of all species stored in closed containers was much better than germination of seeds stored in open containers. (U. S. Regional Pasture Research Laboratory and Pennsylvania State College.)

Reactions to Crown Rust in Festuca elatior and F. elatior var. arundinacea. KREITLOW, K. W., and W. M. MYERS. Inoculation of many plants in the greenhouse corroborated field observations that *Festuca elatior* was generally susceptible to crown rust (*Puccinia coronata*) while *F. elatior* var. *arundinacea* was usually resistant. Of the many collections of *F. elatior* tested, 3 plants from 1 collection were immune from rust. Additional material, which varied in rust reaction from susceptible to immune, from the same and neighboring fields proved to be *F. elatior* var. *arundinacea*. Cytological studies of the species indicate that the small, fine-leaved *F. elatior* has 14 somatic chromosomes while the tall, coarse *F. elatior* var. *arundinacea* is a hexaploid and has 42 chromosomes. Hybrids between the two were completely sterile.

Virus Inhibitors in Spinach Extract. KUNTZ, J. R., and J. C. WALKER. Cucumber virus 1, turnip virus 1, and tobacco virus 1 are not readily recovered from infected spinach by mechanical juice extraction although the first 2 viruses are easily transferred by aphids. Crude spinach extract added in equal proportions to infectious plant juice containing these viruses usually completely inhibits infectivity. Spinach extract contains 2 distinct inhibitive entities. One of these, inhibitive to tobacco virus 1, is thermolabile, nondialyzable, unstable in extremely acid and extremely alkaline solutions, precipitated by alcohol, and adsorbed by some activated charcoals. Infectivity of noninfectious mixtures of spinach extract and infectious juice can be restored by removal or adsorption of the inhibitor. The second inhibitor, inhibitive to turnip virus 1 but not to tobacco virus 1, is thermostable, dialyzable, is not precipitated by alcohol, is not readily adsorbed on activated charcoals. Infectivity of a noninfectious mixture is restored by addition of calcium chloride.

Serological Relationship between Potato Latent Ring-spot and Virulent Ring-spot Viruses. LARSON, R. H. Precipitin and precipitin-absorption tests indicate that the virulent potato ring-spot virus causing a mottle and necrosis in certain varieties of American potatoes in the field is a strain of the latent potato ring-spot virus. No significant difference was detected between the ability of the virulent ring-spot virus or the latent ring-spot virus to induce antibody formation in rabbits; both sera reacted at the same dilution and showed high titre. Reciprocal precipitin tests showed that the potato latent mottle, latent ring-spot, and virulent ring-spot viruses are serologically indistinguishable. There were no detectable differences in isolates of the virulent ring-spot virus from Wisconsin-grown Chippewa, Katahdin, Red Warba, Sebago, or Pontiac varieties and isolates from the Sebago variety from Minnesota, Michigan, or Maine. Veinbanding virus (aphid-transmitted component of rugose mosaic) was not cross-active with the latent mottle, the latent ring-spot, or the virulent ring-spot virus antisera. The virulent potato ring-spot virus is designated as a strain of the potato mottle (latent) virus, ring-spot group.

The Cytology of Ustilago striaeformis from Poa pratensis. LEACH, J. G., and MARY ALICE RYAN. This fungus completes its life cycle in culture on agar media. The germinating chlamydospore does not form a true promycelium but forms a branched germ tube of indeterminate growth. No sporidia are produced. The chlamydospore contains a fusion nucleus that undergoes reduction division soon after germination begins. A chance reassortment of nuclei takes place in the branching germ tube or resulting mycelium, and karyogamy occurs without an intermediate dikaryophase. Some cells may remain in the haploid condition and give rise to haploid mycelium. The diploid cells continue to multiply vegetatively, forming a characteristic fragmenting mycelium that is readily distinguished from the typical radiating haploid mycelium. The diploid mycelium in its typical form of growth breaks up into short fragments, each cell of which contains a single, large, diploid nucleus. A single cell of the diploid mycelium may be transformed directly into a chlamydospore. Thus, the fungus appears to be homothallic, but produces both haploid and diploid mycelium. It has no true dikaryophase.

Control of Cucumber Anthracnose with Fermate. LEBEAU, F. J. Successful culture of fall cucumbers in southeastern Louisiana depends on satisfactorily controlling downy mildew, and although copper compounds often injure the foliage, Bordeaux mixture and various copper dusts have been generally used. In the last 2 years, mildew control was complicated by epidemics of anthracnose, which the standard fungicides did not satisfactorily control. In 1944, only Bordeaux mixture, 4-4-50, and Fermate dust, 10-100, protected the plants from anthracnose. In 1945, when a severe epidemic of anthracnose destroyed many fields by the middle of the harvest season, Fermate, 10-100, completely protected the plants from anthracnose and from downy mildew, without foliage injury. Bordeaux mixture and Dithane were more satisfactory than the copper dusts but did not give satisfactory control during the last quarter of the harvest. Acre yields of 233, 244, 291, and 461 bushels were obtained, respectively, in the plots treated with Bordeaux mix-

ture, 12 per cent tribasic copper sulphate dust, 3 per cent Cuproicide dust, and 10 per cent Fermate dust. Low yields in the Bordeaux mixture plots were attributed to foliage injury.

*Relations of Carbon and Nitrogen Sources and Vitamins to the Growth of Pathogenic and Nonpathogenic Lines of *Venturia inaequalis*.* LEBEN, CURT C. The physiology of 4 wild-type pathogenic and 3 nonpathogenic cultural mutant lines was studied *in vitro*. At 0.5 per cent concentration, 39 carbon sources were tested in a mineral salt-agar medium containing 1 g. per liter of malt extract. The most favorable compounds were cellobiose, dextrin, fructose, glucose, maltose, mannitol, mannose, melezitose, raffinose, and sucrose. Twenty-one nitrogen sources, mostly amino acids, were tested in a similar medium containing glucose and nitrogen equivalent to 0.003 M KNO₃. The best compounds for most lines were arginine, glutamic acid, histidine, proline, urea, NH₄NO₃, (NH₄)₂SO₄, and KNO₃. Ability to utilize the carbon and nitrogen sources varied among the lines. In a mineral salt-glucose medium containing purified agar, the following substances were tested singly and in combination: adenine sulphate, ascorbic acid, *b*-alanine, biotin, calcium pantothenate, choline chloride, a "folic acid" concentrate, guanine, inositol, niacin, *p*-aminobenzoic acid, pimelic acid, pyridoxine, riboflavin, thiamine, uracil, and xanthine. Only thiamine had a beneficial effect, and it was greater for some lines than for others. When thiamine was present, substances in "vitamin-free" casein hydrolysate also stimulated growth. None of the observed differences in the physiology of the lines was clearly associated with their pathogenic capabilities.

Control of Bacterial Pustule of Soybean by Dusting. LEHMAN, S. G. In 1944, favorable results were obtained from an experiment designed to control bacterial pustule of soybean by dusting growing plants with fungicides. The experiment was repeated with amplification in 1945. The dust preparations used were: (1) copper-clay containing 7 per cent metallic copper from Tenn. 34, (2) copper-sulphur having 7 per cent metallic copper from Tenn. 34, (3) copper-talc containing 7 per cent copper from Copper Compound A, (4) 20 per cent Fermate in Pyrox tale, (5) 20 per cent Zerlate in Pyrox tale, (6) 325-mesh sulphur, (7) 10 per cent Fermate in sulphur, (8) 5 per cent DDT in sulphur. Six applications of each dust were made. Only dusts containing copper reduced bacterial pustule. In control rows not dusted, less than 5 per cent of the leaves were entirely free of disease. On the remaining 95 per cent damage ranged from slight to severe. In rows dusted with copper, 37 to 74 per cent of the leaves were entirely free of disease; the remaining leaves showed little injury, few or none being severely damaged. Plots dusted with copper yielded 4.9 bushels per acre more than plots not dusted. Sulphur dust reduced yields about as much as copper increased them.

Field Tests with Dow 9 on Cottonseed. LEHMAN, S. G. From preliminary greenhouse tests on cottonseed treated with hitherto unused preparations, Dow 9, containing 100 per cent of zinc salt of 2,4,5-trichlorophenol, was selected for field trials in 1944. It was compared with New Improved Ceresan, the standard material for cottonseed treatment. Dosages of 1.5, 3, and 6 g. of dust per 1000 g. of seed were used. Dow 9 at the lowest dosage gave seedling stand increases equal to those from Ceresan. Higher doses of Dow 9 gave smaller increases. In 1945, Dow 9A, containing 25 per cent, and Dow 9B, containing 50 per cent of zinc salt of 2,4,5-trichlorophenol, were each used at 1.5, 3, and 6 g. per 1000 g. of seed. New Improved Ceresan increased surviving seedlings 90.3 per cent; 9A, 124.8 per cent; and 9B, 130.7 per cent. The 3 to 1000 doses of both Dow preparations gave increases approximately equal to higher doses. In another test, seed treated with 3 and 6 g. of Dow 9B per 1000 g. of seed and stored 7 and 42 days in grain sacks gave higher seedling survival than Ceresan-treated seed stored for the same periods. Emergence from seed treated with 9B did not differ significantly for the two storage periods.

Outstanding Diseases of Agricultural Crops and Uses of Fungicides in the United States. McCALLAN, S. E. A. To facilitate research on new chemicals as possible fungicides and specifically to select rapid methods for evaluating them in laboratory and greenhouse, it is necessary to know which plant diseases are outstanding on a national basis. A tentative method for determining those diseases is based on an index obtained from the product of the logarithms of the estimated annual percentage loss (from Plant Disease Reporter) and of farm value. The indices, thus obtained, of 36 outstanding diseases are recorded together with average annual losses, 10-year fluctuations, and present major control measures. Tables showing estimated annual consumption of fungicides are arranged according to chemicals, crops, and diseases. Thus may be seen the most outstanding potential uses for new or improved fungicides, and likewise the diseases for which it is desirable to develop test methods. Among certain outstanding diseases there is need for better fungicides: (a) seed treatments—corn and cotton seedling blights, oat smuts; (b) sprays and dusts—apple scab, potato tip burn and late blight, peach and cherry brown

rot, pear blight, peanut leaf spot, tomato blights. Adequate methods for fungicide evaluation are available in certain cases cited, but further development and standardization are necessary for many.

• *A New Helminthosporium Disease of Oats.* MEEHAN, FRANCES, and H. C. MURPHY.

A new species of *Helminthosporium* pathogenic on certain oat varieties was isolated in November, 1944, from a diseased seedling of Tama oats grown in the germinator. Numerous field isolations of this fungus were obtained during 1945. Infected seedlings showed necrosis of first internode, scutellum, and seminal roots. Affected leaf blades were dull bluish-gray, had reddish brown full-length stripes 1 to 3 mm. wide, and their margins withered. Wilting of the leaves and death of severely infected plants followed a few days after the first leaf discolorations. Infected adult plants in the field had a brownish translucence of the lower internodes and tended to break over near the base. Late in the season the nodes were covered with abundant congliphores, and plants ripened prematurely. Normal conidia were light olivaceous, slightly curved, rounded at the base, widest near the center and tapering toward the apex, moderately thin-walled. Germination was polar. The maximum conidial measurements observed were: length, 111 μ ; width, 25 μ ; number of septa, 11. The nodes were 75 μ , 18 μ , and 8 μ , respectively. The organism was isolated frequently also from timothy. (Iowa Agricultural Experiment Station and U. S. Department of Agriculture.)

Soil Actinomyces in Relation to Panama Disease of Banana. MEREDITH, CLIFFORD H. Nine cultures of Actinomyces, isolated from soils of several banana plantations and demonstrated to be antagonistic to *Fusarium oxysporum cubense* in the laboratory, were applied near the stools of banana at planting time in an experimental planting at Orange River, Richmond, Jamaica, B. W. I. The field was infested with the *Fusarium* that causes Panama disease. The Actinomyces were grown in flasks, on guinea grass with soil and water. There were 15 plants in each plot and 7 replicates of each treatment: Actinomyces from other fields, Actinomyces from other fields plus grass mulch, Actinomyces from Orange River field plus grass mulch, grass mulch alone, and the check with no treatment. At 5 months after treatment the banana plants treated with Actinomyces were larger than check plants. At 12 months there were no statistically significant differences in the Panama disease in the plots.

Some Phenols as Agricultural Fungicides. MEULI, LLOYD J., and BERNARD J. THIEGS. Results of greenhouse and field tests since 1943 indicate that dust compositions of zinc 2,4,5-trichlorophenolate (Dow Seed Fungicide 9A and 9B) for seed treatment effectively control certain seed-borne pathogens and protect against various soil-inhabiting organisms that cause preemergence damping-off. The 2,4,5-trichlorophenol was more effective than 2,4,6-trichlorophenol. Of the several salts tested as seed fungicides, the zinc salt of 2,4,5-trichlorophenol was most consistently effective in producing high seedling emergence. Testing has been most intensive on cotton and peas, but results also show that the treatment has value for many other agricultural seeds. The chemical is heat-stable and is difficultly soluble in water and the usual organic solvents.

Relation of Sour Cherry Yellows to Yield. MOORE, J. DUAIN. For several years comparative yield records were taken on yellows and yellows-free trees in 2 commercial orchards in Door County, Wisconsin. One orchard was 21 years old, the other 13, in 1945. Both were surveyed for occurrence of yellows before yield records were taken and annually thereafter. The yellows trees of each orchard were classified according to the year in which the disease was first recorded. Each diseased tree was paired with an adjoining, comparable, yellows-free tree. Yield data from the 2 orchards, recorded in pounds per tree, were very similar. Though individual trees varied considerably in the rate and extent of reduction in fruitfulness, the general trend was for little or moderate reduction in the first or second year following first observation of symptoms and a greater reduction in the ensuing 2- or 3-year period. In 1945, in the 13-year-old orchard, for the group of trees known to have been diseased 5 years or more the average reduction in yield was approximately 50 per cent (16 pairs of trees); in the 21-year-old orchard, approximately 62 per cent (53 pairs of trees). On the average the trees in the older orchard had been diseased longer.

Relation of Temperature to Expression of Symptoms of Sour Cherry Yellows and Necrotic Ring Spot. MOORE, J. DUAIN, and G. W. KEITT. Potted Montmorency cherry trees were budded from 3 sources of sour cherry yellows and placed in greenhouses with approximately constant temperatures of 16°, 20°, 24°, and 28° C., respectively. Two trees budded from each source were placed in each greenhouse. During 2 greenhouse seasons, yellows leaf symptoms were expressed only at 16° C. In the third season all

trees were placed at 16°, and all except 1 that had been at 28° for 2 seasons expressed yellows symptoms. Potted Montmorency trees budded from a source of necrotic ring spot and put in controlled temperature greenhouses expressed necrotic ring-spot symptoms over the entire range from 16° to 28°. Symptoms were expressed more rapidly and with more necrosis at the higher temperatures. Best leaf-symptom expression occurred at 20° or 24°. In greenhouse experiments with *Prunus pumila*, *P. virginiana*, *P. americana*, and *P. besseyi* budded from a source of necrotic ring spot, symptom expression was either very poor or lacking at night temperature of approximately 10° C. and day temperature of 16° to 24° C. Symptom expression occurred on all 4 hosts and was more general and conspicuous at 24° to 28° C., day and night.

Spraying and Dusting Potatoes in Michigan in 1945. MUNCIE, J. H., and W. F. MOROSKY. At Lake City, Michigan, potatoes sprayed with Bordeaux DDT 8-12-1-100 produced significantly higher yields than those sprayed with Bordeaux 8-12-100 alone and those sprayed with the fixed coppers, Tribasic copper sulphate, Dow F-48, copper oxychloride sulphate, Copper Compound A, and Yellow Cuproicide, to which had been added 0.5 lb. zinc sulphate and 1 lb. hydrated lime per 100 gal. Plants sprayed with fixed copper plus Z-39 (dichloro diphenyl dichloro ethane), Pyrethrum, B-72 (beta beta dithio cyano ethyl ether), Dithane, or Zerlate yielded significantly less than those with Bordeaux DDT 8-12-1-100 or Tribasic DDT. Indications were that the excess lime in the Bordeaux had no deleterious effect upon DDT and that excess lime in itself did not cause decreased potato yields when leafhoppers or other leaf-feeding insects were reduced to a minimum. Leafhopper control was best with DDT followed by Pyrethrum, B-72, Z-39, and nicotine sulphate. Tests of 22 dusting materials were inconclusive because of drifting of dusts containing insecticides for control of leafhoppers. In spray tests in 9 counties of the Upper Peninsula of Michigan 2 fixed coppers plus DDT were compared with Bordeaux 8-12-100 and fixed copper zinc sulphate-lime. Addition of DDT increased yields over Bordeaux or fixed copper-zinc sulphate-lime and decreased markedly leafhopper, flea-beetle, and aphid populations.

* *Reaction of Oat Varieties to a New Species of Helminthosporium.* MURPHY, H. C., and FRANCES MEEHAN. Oat varieties possessing the "Victoria type" of crown-rust resistance were highly susceptible in the field and greenhouse to a new species of *Helminthosporium*, while varieties lacking this "Victoria" rust resistance were resistant. No exceptions to this were observed among 248 oat selections tested in the greenhouse. Named Victoria-hybrid selections such as Boone, Tama, Vichand, Cedar, Osage, Neosho, Forvie, Fultex, and Letoria were susceptible. The Rond-hybrid selections, Clinton, Benton, Eaton, Bouda, Minto, and Camellia, were resistant, as were older varieties such as Iogold, Richland, Gopher, Huron, Fulghum, Marion, Erban, Vanguard, Lee, Fulwin, and Red Rustproof. F₂ populations of 8 hybrids, each involving one Victoria crown rust resistant parent, segregated 3 susceptible to 1 resistant when inoculated with the *Helminthosporium*. Other populations of these crosses segregated 3 resistant to 1 susceptible to race 45 of crown rust. These data suggest complete linkage for the genes controlling "Victoria type" of crown-rust resistance and susceptibility to the *Helminthosporium*. This fungus has been isolated from Victoria-hybrid selections grown in Texas, Iowa, Pennsylvania, North Dakota, and Montana in 1945. At the Iowa Experiment Stations, Osage, Victoria × Rainbow, and (Victoria × Richland) × Bannock were reduced 50 per cent in yield by the attack of the fungus. Seed-borne infection was effectively controlled with New Improved Ceresan. (Iowa Agricultural Experiment Station and U. S. Department of Agriculture.)

Relative Effectiveness of Certain Organic and Inorganic Fungicides for the Control of Phytophthora infestans on Potatoes. NAGEL, C. M. Excellent control of epiphytotic late blight was obtained with 4 of the 8 fungicides tested. Bordeaux, Yellow Cuproicide, Phygon (2,3-dichloro 1,4-naphthoquinone), and Dithane (disodium ethylene bisdithiocarbamate) afforded uniformly low percentages of disease from 3 foliar spray applications. Phygon, at 1.5 pounds per 100 gal. water, increased yields by 70.3 per cent when compared to the check. Yellow Cuproicide, Dithane, and Bordeaux, although as effective as Phygon in disease control, produced smaller increases (44.3, 16.7, and 14.9 per cent, respectively). Zerlate (zinc dimethyl dithiocarbamate) and Copper Compound A produced increases in yield of less than 10 per cent. Small decreases in yield were obtained with Isothian Q15 (lauryl isoquinolinium bromide) and Puratized N5E. A randomized split-plot design with 3 replications was employed in this experiment with Early Ohio potatoes. One half of each plot was treated with a dust of DDT, 2,2-bis (p-chlorophenyl) 1,1,1-trichloroethane, for the control of the 3 major insects common to potatoes in South Dakota, namely, leafhopper, flea beetle, and the Colorado potato beetle. Ten sweeps yielded an average of less than 6 insects per plot; hence, differences in disease control and yields are very largely attributable to the relative effectiveness of the various fungicides. All fungicides were applied as sprays at the recommended dosages.

Availability of Copper in Dust as Influenced by Various Diluents. NIKITIN, A. A. There are several inert diluents available for use in pesticidal dust mixtures. There is considerable variation in chemical and physical properties of these diluents, which have an effect on the rate of flow, adherence, and effectiveness of the final dust mixtures for crop protection. Emphasis was placed in this study on adherence and availability of soluble copper. This availability depends greatly upon the sorption capacity of various diluents. Results secured from these tests show that a certain correlation exists between surface tension of the water suspension of diluents and their sorption capacity for soluble copper. Study on the sorption capacity of copper by various diluents shows that pyrophyllite clay absorbs much less copper than Eastern Magnesite and especially Loomkill. This sorption capacity is in direct relation to the base exchange capacity of the diluent. Zinc salts are more readily sorbed by diluents than copper and can be used to increase the amount of soluble copper available for the control of heavy infestation. Neutral sodium salts reduce the concentration of available copper due to increased sorption capacity of the diluent.

Use of D-D Mixture Permits Two Crops of Watermelons per Year in Breeding Program. PARKIS, G. K. Applying D-D mixture to Norfolk sand known to contain *Heterodera marioni* and suspected of containing the meadow nematode, immediately following a spring crop of melons, permitted growth to maturity of a second crop of melons (August to September) at Leesburg, Florida. Five cc. of D-D per hole was applied in holes 6 inches deep, 12 inches apart in rows that were 12 inches apart. Approximately 15 square feet of soil were treated per hill. With roughly 1200 hills per acre, the actual quantity of D-D used was 175 pounds per acre. The soil temperatures, 6 inches deep, ranged from 81° to 90° F. in the day to 78° to 82° F. at night. There was no evidence of injury to seeds planted 10 days after soil treatment. On nontreated soil the survival of *Fusarium*-resistant Blacklee was 25 per cent and most plants died when runners were 3 feet long. On treated soil, 69 per cent of Blacklee lived and 72 per cent hybrid plants survived. No fruits were obtained from plants on nontreated soil. Growing 2 crops of melons a year will speed up markedly the present program of breeding for resistance to cucurbit anthracnose and downy mildew.

Occurrence and Importance of Cankers and Rots in Deciduous Forests in Quebec. POMERLEAU, RENÉ. Surveys in several localities in Quebec were made between 1942 and 1944 to obtain information on the health of deciduous forest trees. At the Forest Experimental Station at Duchesnay, 3147 trees distributed in 115 sample plots were studied. The data obtained were completed by surveys in sugar maple groves in 17 localities, and by stem analysis of 279 trees in 4 sample plots in a white birch stand. At Duchesnay, 13.9 per cent of the trees were infected with at least one canker, and 21 per cent were more or less severely rotted. Close examination of 930 trees in maple groves showed that 22.1 per cent of them were cankered and 18.4 per cent rotted. In the white birch stand, 9.5 per cent of 279 trees were cankered and 14.3 per cent rotted. In all these stations, *Nectria* canker was the most prevalent and injurious, especially to maple, white birch, and yellow birch, and the incidence of infection varied with site. *Entypella* canker occurred on 3 to 8 per cent of the maples, especially on the young trees. *Diatrype macounii*, *Poria punctata*, and *Pholiota squarrosoides* were found for the first time to be responsible for canker. *Fomes igniarius* caused the most widespread rot in maple, birch, and aspen, but other fungi, taken together, produced comparable losses.

Variability in Phoma lingam. POUND, GLENN S. A *Phoma* species has been found as a pathogen of cabbage and Chinese cabbage seed plants in the Puget Sound area of Washington, causing minor leaf-spot and stalk-rot injury. It is morphologically similar to *Phoma lingam* (Tode) Desm., produces typical black leg on members of *Brassica oleracea* L., attacks a wide range of cruciferous plants, and differs only slightly in pathogenicity from *P. lingam*. Distinct strains of monosporic origin have been obtained from *P. lingam*, these differing in type and rate of growth, staling, and amount of sporulation. Only slight differences in conidial size occur between these strains, but marked differences in shape and size of pycnidia are found. Slight differences in pathogenicity have been observed. Numerous saltants tend to link the strains. Because of the extent of variability in *P. lingam*, the Puget Sound isolate is considered tentatively to be a variant of this species. (Washington Agricultural Experiment Stations, Vegetable Seed Production Laboratory, Mount Vernon, Washington.)

Cabbage Varietal Reactions to Mosaic Viruses. POUND, GLENN S. For the past 3 years commercially important cabbage varieties have been tested for their relative susceptibility to mosaic viruses. In experiments in which relative susceptibility was determined by a disease-index system the varieties tested showed a distinct gradient, but they could be divided roughly into 3 groups. In the least susceptible group were Wisconsin All Sea-

sons, Stein's Flat Dutch, All Head Select, Succession, and Globe. In the intermediate group were Jersey Wakefield, Charleston Wakefield, All Head Early, Copenhagen Market, Golden Acre, Marion Market, Midseason Market, Glory of Eukhuizen, Round Dutch, and Premium Late Flat Dutch. The most susceptible group contained the ballhead varieties, namely Wisconsin Hollander, Rugner, Penn State Ballhead, Wisconsin Ballhead, and Hollander. The first 3 in the last group were only moderately susceptible through mid-season but developed very severe symptoms by the time heads were mature. The relative susceptibility of the various varieties was about the same when either the cabbage A strain or the cabbage black-ring strain of *turnip virus 1* was combined with the cabbage B strain of *cauliflower virus 1* to produce the mosaic disease. (Washington Agricultural Experiment Stations, Vegetable Seed Production Laboratory, Mount Vernon, Washington.)

Varietal Reaction of Cotton to Bacterial Blight. RAY, W. W. Approximately 250 varieties and selections of cotton have been tested in the seedling stage to determine their reactions to the bacterial-blight organism. This technique involved soaking acid-delinted seed in a bacterial suspension, planting this seed in moist sand, harvesting the seedlings one week later, and sealing the disease intensity by means of a disease index based on the number of healthy and diseased cotyledons and the nature of the lesions. The results obtained by means of this rapid technique showed good correlation with those obtained by inoculating older plants in the field. With but few exceptions, commonly grown commercial varieties were the most susceptible to blight. Certain lines such as Stoneville 20, Stoneville 62-1, Stoneville 462, and Oklahoma Triumph 92-1 had considerable resistance. The artificial inoculation of seedlings and field plants to determine varietal reaction to blight is proving very useful in cotton breeding.

A Rusty Mottle-like Virus Disease of the Sweet Cherry in Utah. REEVES, E. L., and B. L. RICHARDS. A virus disease of sweet cherry resembling rusty mottle occurs in destructive proportions in Utah orchards. Incidence of transmission by bud graft is high, approaching 100 per cent. The virus rapidly becomes systemic in the tops of inoculated trees and induces in sequence retarded blossoming and leaf development in the spring, brown necrotic leaf areas with shot hole effect, early senescence and extensive defoliation, rust-colored chlorotic spots, premature autumn senescence, roughening of bark, and bud killing. Rangy limbs with scant foliage, usually confined to terminal portions of branches, ultimately result. In early breakdown of chlorophyll resulting in ring-spot and line patterns, in early leaf senescence and subsequent defoliation prior to fruit ripening, the disease in Utah follows the same sequence as and resembles essentially rusty mottle described for Washington. In Washington, leaf necroses, pronounced devitalization of the tree, blossom and leaf retardation, and bark roughening do not occur. Consideration has been given to the possibilities that (1) the virus in Utah is a more virulent form, (2) the disease in Utah results from a combination of viruses including the rusty mottle virus, (3) the environment influences expression, (4) a different virus is involved.

Wilt and Decline, a Virus Disease of Sweet and Sour Cherries in Utah. RICHARDS, B. L., E. L. REEVES, and LEE M. HUTCHINS. "Wilt" and "dieback," common diseases in the sweet and sour cherry, have long been observed in Utah orchards. These well-known diseases assume additional significance because buds taken from red-leaf-diseased chokecherry plants and grafted into sweet and sour cherry varieties on Mahaleb rootstock induce a characteristic cherry wilt during the season following inoculation. A definite decline occurs in trees that survive the wilt. The wilt and decline, artificially produced, resemble in many respects the diseases observed in the orchards. Decline is progressive and, although some of the inoculated trees die within 2 years, others survive for a number of years. Evidence indicates that both wilt and decline result either from the direct effect of the virus or from the effect of products of the disease on the Mahaleb rootstock. The virus failed to pass from diseased sweet cherry branches through Mahaleb understock to healthy branches of the sweet cherry separately top-worked on Mahaleb. Red leaf in the chokecherry has been experimentally connected with the form of Western X disease of the peach in Utah. A relationship between the cherry wilt and Western X in peach appears to be demonstrated.

Effect of Soil Nutrients on the Development or Suppression of the Leaf-roll Symptom in the Green Mountain Potato Grown in the Greenhouse. RICHARDS, M. C., R. C. JONES, and STUART DUNN. From December to March, when days are short and frequently cloudy, the leaf-roll symptom can be masked in the Green Mountain variety of potato grown in the greenhouse by adding to the soil at planting time certain soil nutrients. When tubers with severe net necrosis were planted in 6-inch pots to which 11 g. sodium nitrate or 11 g. of 8-16-16 fertilizer had been added, the leaf-roll symptom was absent in from 75 to 100 per cent of the plants. Plants from sections of the same tubers planted in pots with 11 g.

superphosphate or 11 g. potassium chloride had well-developed symptoms in 87 to 100 per cent of the cases. Plants in the pots containing nitrogen or complete fertilizer showed a higher percentage of masking when the soil was kept relatively dry. The masking was less when the tests were repeated in April and May, and no masking occurred when similar tests were made in the field during the summer. There appears to be a relationship between light conditions and nitrogen nutrients with respect to the suppression of the leaf-roll symptom in the Green Mountain potato.

Geographic Distribution and Control of Tilletia spp. in Mexico. RODRIGUEZ V., JOSÉ. Bunt is second to stem rust in importance as a disease of wheat in Mexico. Losses up to 40 per cent have been recorded in fields where nontreated seed was sown. While the disease occurs wherever wheat is grown in Mexico, greatest losses occur in the northern wheat-growing region. *Tilletia levis* comprised 87.09 per cent of collections made in 1945 and was found in all parts of Mexico where wheat is grown. *Tilletia tritici*, which represented 12.91 per cent of the 1945 collections, has been found only in southern Mexico. In this area spring and durum types of wheat predominate while in the North, spring types, semi-winter, and winter types occur in that order. Regional variety tests are in progress, a seed treatment campaign is getting under way, and improved varieties are being developed.

Observations on Leaf and Stripe Rust of Wheat in Mexico. RODRIGUEZ V., JOSÉ. Field tests in central Mexico demonstrated that a number of improved U. S. wheat varieties were highly susceptible to the races of leaf rust (*Puccinia rubigo-vera*) in Mexico. Pilot, Rival, Regent, Renown, and Newthatch were susceptible. Mida was resistant. Several selections of Kenya were moderately susceptible, and three durums, Mindum, Kubanka, and Arnautka, rusted rather heavily. Carlton and Stewart were resistant. Most of the local varieties were susceptible to leaf rust. Stripe rust (*Puccinia glumarum*) attacked many local wheats during late winter or early spring when they were heading. Some of the Kenyas also were susceptible as were many lines of Renacimiento × (41-116). Lines from Trigo Supreme × (41-116) were resistant.

A Dwarfing and Witches' Broom on Corn in Iowa. SEMENIUK, G., I. E. MELIUS, J. R. WALLIN, C. L. GILLY, and MURIEL O'BRIEN. A dwarfing, branching, and witches'-broom disease on maize has occurred sporadically in Iowa during the last 20 years. This past season, 1945, it was more common than at any previous time. In some low lands in the north central part of the State, from 2 to 50 per cent of the plants were diseased. Many hybrids were affected. The dwarfed plants were often less than 2 feet tall and excessively branched. In some cases the plants were only a foot tall and dead when healthy corn was in the milk stage. In certain other cases the plants were not stunted but were excessively tall, with almost twice the normal complement of leaves and with few branches. Numerous witches' brooms were produced by the terminal meristems of the side branches and the floral organs. Leafy shoots developed from the floral organs. Often these witches' brooms were on partially dwarfed and much-branched plants. These symptoms of dwarfing and excessive branching were like those caused by *Sclerospora graminicola* and already described on corn and other hosts. This pathogen was prevalent on *Setaria viridis* in the cornfields. Weather conditions in the spring were cool and wet, favoring *Sclerospora* infection.

Studies on the Milo Disease. SLAGG, C. M., and L. E. MELCHERS. Laboratory and greenhouse studies on the root rot of milo, or "milo disease," from 1939 to 1943 indicate that fungi other than *Pythium arrhenomanes* Drechs. may be mainly responsible as causal agents. Pure cultures of *P. arrhenomanes*, *Fusarium moniliforme*, *Corticium vagum*, and other fungi isolated from diseased milo plants, were separately increased in steamed soils which were later planted with seed of resistant and of susceptible strains of milo. The comparative growth of resistant and susceptible strains in any soil was used as a measure of the severity of the root rot in that soil. Appearance of the milo disease in steamed, noninoculated soils led the writers to suspect seed transmission, and this was proved experimentally. In 1941, 22 different lots of milo seed grown in 1939 and 1940 at Garden City and Ft. Hays, Kansas, were tested, and 8 of these seed lots transmitted the milo disease. In 1942, 27 different seed lots were tested, of which 10 transmitted the milo disease. One seed lot more than 3 years old infested soil in which it was sown.

Studies in the Northern Great Plains upon the Effect of Crop Sequencer on Root-rot Losses in Cereals and Grasses. SPRAGUE, RODERICK. Field and greenhouse studies have shown that crop-rotation practices have had certain effects on the parasitic activity of *Pythium arrhenomanes* Drechs., *P. debaryanum* Hesse, and *Helminthosporium sativum* Pam., King and Bakke on small grains and grasses in western North Dakota during the favorable crop years of 1940-45, inclusive. Ail but *P. debaryanum* were greatly inhibited

after several years of continuous fallow but one-year fallow did not appreciably reduce injury from these root-rot fungi. Wheat after corn, or wheat after oats after fallow aided in holding root-rot losses to a moderate level. Barnyard manure reduced root rots only during years of abundant precipitation, as did green manure crops. The use of grasses in long-term rotations was of value in reducing losses from root rots during the grain crop years, provided corn or some crop other than small-grain followed breaking. Rotation practices that maintain soil fertility as much as possible aid the crop plant in its competition with the soil fungi for nutrients. (U. S. Department of Agriculture and North Dakota Agricultural Experiment Station.)

Adaptation of Monosporidial Lines of Ustilago zeae to Arsenic. STAKMAN, E. C., FRANK V. STEVENSON, and C. T. WILSON. Nonmutable as well as mutable haploid monosporidial lines of *Ustilago zeae* increased their tolerance for sodium arsenite from an initial tolerance of 2400 ppm. to 7000 ppm. during 10 transfer generations on arsenic media. Ability to grow on the same concentration of arsenic also increased with successive transfers. At 3000 ppm. the colony size increased from 15 to 28 mm. as a result of successive transfers. The appearance of colonies on arsenic media was different from that on potato-dextrose agar. When arsenic-adapted lines were returned to arsenic-free media they tended to revert to the original cultural type and to lose their tolerance for arsenic. At the end of 4 transfer generations on potato-dextrose agar they had reverted to the original in all respects, although initial growth still was slightly slower than that of cultures that had been on potato-dextrose agar continuously.

A New Method of Inoculating Some Maydcae with Ustilago zeae (Beckm.) Unger. STEVENS, KAY, I. E. MELHUS, G. SEMENIUK, and LOIS TIFFANY. Corn, germinated on moist blotters and with plumules one-half to one and one-half cm. long, were inoculated with sporidial suspensions from 2 monosporidial lines of *Ustilago zeae*. The lines, grown separately in carrot decoction, were mixed and injected by hypodermic needle into the plumule slightly above the coleoptile node. Infection appeared within 5 days on inoculated seedlings held at 30° C. and 90 per cent relative humidity. Well-developed galls and dwarfing of plumule were evident 2 days later. The 115 collections of inbred lines, hybrids, and open-pollinated varieties from the United States averaged 96.5 per cent infection; 165 collections of Mexican and Central American open-pollinated corns averaged 88 per cent; several collections from Central America had as low as 50 per cent infection. When the mixed inoculum was diluted with distilled water the infection percentages were lower than with undiluted inoculum. Injection of inoculum into the first internode of the corn seedling failed to yield smut. *Euchlaena mexicana* was susceptible to *U. zeae*, but *Tripsacum dactyloides* and *Coir lachryma-jobi* were not. Corn and teosinte were susceptible to *U. dieteliana* obtained from *Tripsacum*. These plants at flowering developed sori like those found on *Tripsacum* and similar to those formed on sugar cane by *U. scitamina*.

A Correlation between Pigment Production and Pathogenicity Among the Actinomyces Causing Scab of Potato. TAYLOR, CARLTON F., and PHARES DECKER. In two consecutive studies, one on the cultural characteristics and one on the pathogenicity to potato of a large number of isolates of the genus *Actinomyces* (mostly from scabbed potatoes), there was a positive correlation between pigment production on milk tubes and pathogenicity to potatoes. Many of the isolates produced, within a few days, a dark brown ring on skim milk cultures. The color of this ring was within the limits of 15 i-m to 17 i-m (Ridgway). Of the 127 isolates common to both studies, 61 produced a dark brown ring, while 66 did not. All of these 61 isolates caused typical scab of potato in controlled greenhouse trials, while the other 66 did not. With a number of the latter, soil inoculation was followed by superficial russetting of the surfaces of some of the tubers. This russetting did not resemble typical scab of potato, and in no case was it possible to re-isolate the organism introduced as inoculum.

Pathogenicity of Isolates of Rhizoctonia solani on Soybean. TERVET, IAN W., and C. T. TSIANG. Ten varieties of soybean were inoculated with 8 isolates of *Rhizoctonia solani* in steamed soil in the greenhouse at 70° F. The isolates differed considerably in virulence and the soybean varieties differed greatly in susceptibility. One isolate from sweet clover was strongly pathogenic on most varieties. It caused severe pro emergence killing in the varieties Bansei and Taystee but relatively little in the varieties Habaro and Kabbott, although severe stem lesions eventually developed on most plants and there was considerable rotting of tap roots and laterals. Isolates from flax and sugar beets were less virulent than the sweet-clover isolate, although they caused poor stands of the Bansei and Taystee varieties. Plants with cotyledons rotted away to leave "bald head" seedlings were not uncommon in all varieties planted in inoculated soil. Such abnormal

seedlings were rarely found in steamed soil. Although isolates of *R. solani* differed decidedly in virulence, there was no evidence of race-host specificity. Varieties of soybean differed in their susceptibility but all varieties were attacked more severely by strongly pathogenic races of *R. solani* than by weaker isolates. Host reaction, as it is used in the separation of races of the rusts and smuts, is not applicable to such a fungus as *R. solani*. (U. S. Department of Agriculture and Minnesota Agricultural Experiment Station.)

Nutrient Requirements of an Antibiotic Soil Fungus, Streptomyces griseus (Krausky) Waksman and Henrici. THORNBERRY, H. H. Excellent growth of *Streptomyces griseus* and good production of streptomycin in surface culture were obtained on a liquid synthetic medium (glucose, 7.4 g.; KH_2PO_4 , 2.38 g.; $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 5.65 g.; NH_4 lactate, 5.4 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.98 g.; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0115 g.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0111 g.; CuSO_4 , 0.0064 g.; and $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.0079 g. per liter at pH 6.95). By varying the concentration of each constituent with other components constant at these amounts, the optimal concentration of each for maximal production was: glucose, 0.04 M. (106 units per ml.); potassium phosphate buffer, 0.05 M. (35 units); NH_4 lactate, 0.05 M. (38 units); MgSO_4 , 0.01 M. (108 units); ZnSO_4 , 5×10^{-4} M. (47 units); FeSO_4 , 5×10^{-5} M. (82 units); CuSO_4 , $1 \cdot 10^{-6}$ M. (55 units); and MnCl_2 , 5×10^{-3} M. (93 units). A new synthetic medium with the constituents at concentrations of these optima gave 60 units of streptomycin in shaker flask and 162 units in surface culture. On beef-extract medium, production of 150 units of streptomycin and excellent growth were obtained at 30° C. At 35° C. growth was excellent but streptomycin production was nil.

Linkage Relationships of a Gene Determining Susceptibility to a Disease in Corn. ULLSTRUP, A. J., and A. M. BRUNSON. Susceptibility in corn to infection by *Helminthosporium carbonum* race 1, is inherited as a monogenic recessive. The genic pair is designated as *Hm hm*. Susceptible inbred lines of corn were crossed with a series of translocation stocks in which the gene *su* was a marker for chromosomal interchange. The F_1 progenies were backcrossed with double recessives. Progeny seedlings from kernels separated on the basis of endosperm characters were inoculated in the greenhouse. In a backcross progeny involving the interchange T1-1a, 1818 seedlings segregated as follows: 664 *su Hm*; 176 *su hm*; 150 *Su Hm*; 703 *Su hm*. The significant deviation of this ratio from the 1:1:1:1 ratio expected in independent inheritance suggested that the genic pair be located on either chromosome 1 or chromosome 4. Progenies involving interchanges of chromosome 4 with other linkage groups gave typical 1:1:1:1 ratios, eliminating chromosome 4 as a possible carrier. Further studies were with linkage groups of chromosome 1. The F_1 progenies (*br f_1*) (*hm+*) were backcrossed to the triple recessive (*hm br f_1*) and backcross progenies were inoculated in the field. Segregation in 16 single-cross progenies was as follows: Parental combinations—1346 (*hm+*) + 1475 (*br f_1*), Region 1 = 377 (*++*) + 228 (*hm br f_1*), Region 2 = 257 (*hbr+*) + 45 (*hm+ f_1*), Region 1 and 2 = 35 (*++ f_1*) + 175 (*hm br-*). The genic pair *Hm hm* probably is situated on chromosome 1 and about 20 cross-over units to the left of the pair *Br br*.

Breeding Tobacco Varieties Resistant to Mosaic. VALLEAU, W. D. There are 2 sources of resistance to mosaic, the N or *Necotiana glutinosa* factor, and the a_1a_2 factors of Ambalema. The N factor when introduced into tobacco localizes the virus in necrotic spots if infection is light or may result in systemic necrosis if infection is heavy. Two satisfactory NN varieties of burley, Ky. 52 and Ky. 34, are being grown extensively in Kentucky with no injury from mosaic. Attempts to convert Ky. 16 to an NN variety by crossing it with *N. glutinosa* and then making as many as 9 backcrosses with Ky. 16 have resulted in NN varieties somewhat earlier and lower yielding than Ky. 16 but otherwise satisfactory. By crossing *Holmes* NN Samsoun with the susceptible, dark, fire-cured varieties Little Crittenden, Little Orinoco, and Brown Leaf, and the air-cured variety One Sucker, and then backcrossing 4 or 5 times, resistant varieties have resulted that appear identical with the original varieties. The use of these resistant varieties is increasing rapidly in 2 counties. In over 10 years' breeding no satisfactory varieties of burley or dark fire-cured tobacco containing the Ambalema factors for resistance have been developed. Plant type is not satisfactory, and the leaves are inclined to wilt and scald under conditions where other varieties are not injured.

Prevalence of Helminthosporium turcicum, Angiospora zeae, and Phyllacora graminis on Corn in Guatemala. WALIAN, J. R. These pathogens caused foliage diseases on corn in the mountainous regions of Guatemala and were destructive on teosinte in Rio Azul and Rio Huista valleys at San Antonio Huista and Jacaltenango and at Laguna Retana near Progreso. *Helminthosporium* often partially or wholly killed leaves before corn was well denting, fruiting profusely on dead areas in the rainy season. *Angiospora* caused local

lesions only on leaf blades where uredial and telial stages were produced in succession, lesions becoming confluent and killing portions of leaves. *Phyllacora* leafspot was prevalent but not so destructive as *Helminthosporium* and *Angiospora*. In 1944 and 1945, when 338 lots of corn from Mexico, United States, and Guatemala were grown at 5000 feet, there were sharp differences in resistance to all 3 pathogens. In 1945, 30 lots of corn from Guatemala, 3 from Mexico, and 2 from United States were very resistant to *Helminthosporium*. Thirty Guatemalan, 13 Mexican, and 10 United States lots were resistant to *Angiospora*. Twenty-three lots from Guatemala were resistant to *Helminthosporium* and *Angiospora*. Most corns were very resistant to *Phyllacora*, but some were markedly susceptible. Gathering leaves for forage as ears mature and removing crop refuse from fields before planting may tend to hold the organisms in check.

Bacterial Blight of Cotton as Affected by the Relative Development of Leaves and by the Method of Inoculation. WEINDLING, RICHARD. Reactions of individual leaves are greatly affected by the stage of their development at the time of inoculation with *Xanthomonas malvacearum*. When mature leaves were sprayed with suspensions of this bacterium, invasion depended primarily on degree of stomatal opening. Mature leaves had a larger number of lesions than younger leaves with fewer functional stomata; however, individual lesions on expanding and bud leaves were very severe, while those on older leaves were progressively smaller. Two types of reaction may be distinguished in susceptible plants inoculated by various methods. (1) Epiphytotic forms of the disease were produced by forceful spraying at close range when stomata were open. Mature, expanding, and bud leaves became heavily infected, but leaves in the barely mature and late expanding stages were the more severely affected. (2) Milder forms of the disease were produced by applying the inoculum either with forceful spray when stomata were closed, or by using little or no pressure. Infection thus induced was the more serious in the expanding and bud stages, and was often more severe when plants were placed in a moist chamber for two days after inoculation.

Cuticle Cracking in Green Tomato Fruits. YOUNG, P. A. Very small cracks that were visible only with reflected light developed abundantly in the cuticle of green tomato fruits, at the stage usually picked for the green-wrap trade. These cracks were found in fruits in the fields at Jacksonville, Texas, within 1 day after 1.38 in. of rain fell on June 22 and 23, 1945, when temperatures were 77° to 90° F. Most of these cracks occurred as segments of concentric circles over the stem end, occupying only the dark green tops of the fruits. Cracks were from 25 to 3000 microns long, 15 to 45 microns wide, and 100 to 300 microns apart. They resulted from spherical expansion of the fruits, and the long cracks commonly contained rows of smaller perpendicular fissures. The cracks remained hyaline until they became deep enough to allow the epidermal cells to dry and then turn black or brown. Within 5 days, the areas with numerous cuticle cracks became sunken and black, making spots $\frac{1}{4}$ to $\frac{1}{2}$ inch in diameter. Tomato buyers graded out all of the fruits with cuticle cracks and severe loss was sustained by local growers.

JAMES PETER JOLIVETTE

1915-1945

J. C. WALKER

James Peter Jolivette was born near LaCrosse, Wisconsin, on July 20, 1915, the son of Bert A. and Vanetta Jolivette. After completing his secondary school training at LaCrosse High School in 1933, he entered the University of Wisconsin in the autumn of that year. Early recognition



JAMES PETER JOLIVETTE
1915-1945

of his ability came in the form of the Steenbock Scholarship Award, which is granted annually to a Senior in the College of Agriculture outstanding in scholarship, leadership, and promise. He received the Bachelor of Science degree in Agriculture, with a major in Soils in June, 1937. Enter-

ing the Graduate School in the same institution in September, 1937, he completed his Doctorate, with a major in Plant Pathology and a minor in Botany, in June, 1941.

In the spring of 1941, Jolivette assumed immediate charge of the truck crop disease field laboratory in southeastern Wisconsin, left vacant by the death of Dr. Otis C. Whipple on February 13 of that year. He was appointed Instructor in Plant Pathology in July, 1941.

Having been a member of the Reserve Officers' Training Corps, as an undergraduate, Jolivette held a commission as Second Lieutenant in the Officers' Reserve Corps, U. S. Army, from the time of his graduation in 1937. Continuing his training in ORC during the period of his graduate studies, he received a promotion to First Lieutenant in July, 1941. Early in 1942 he was called into service and reported for active duty on March 20, 1942. After three months in the Officers' Training School at Fort Benning, Georgia, he was assigned to Company L, 20th Infantry, 6th Division, U. S. Army. In May, 1943, he became Captain of that Company and continued in that capacity until his death. After about a year of intensive training in this country his Division left the Continental United States in September, 1943. Following a brief stay in the Hawaiian Islands, his Company entered the New Guinea campaign early in 1944. During this campaign he was awarded the Silver Star. On January 9, 1945, American troops invaded Luzon, P. I., at Lingayen Gulf and Company L participated in this landing. On February 2, 1945, Captain Jolivette was killed by enemy fire at Munoz, P. I., while conducting a volunteer detachment in the rescue of wounded men and officers. Burial was in the Santa Barbara cemetery near by. He was awarded posthumously the Purple Heart Decoration and a second Silver Star citation.

During his scientific career, Jolivette was engaged in research on vegetable diseases. He carried out very creditable work in the boron deficiency diseases of beet, cabbage, and other vegetables. He studied critically the histological changes brought about in growing plants which were deficient in boron and took an active part in disease resistance breeding programs under way with cabbage, tomato, and bean.

As an investigator he showed marked ability and originality. He took the lead among his associates in the application of modern statistical methods to the design of pathological experiments and to the analysis of results. He showed unusual strength in making contacts with growers and vegetable canners. His field of science has been deprived of a recruit who had won his spurs as an investigator and showed great promise both in fundamental research and in its applications to the agricultural industry.

Captain Jolivette was married on August 23, 1939, to Eloise Lauson. He is survived by his wife and by two sons, Peter Lauson, born May 27, 1941, and David James, born December 11, 1942.

A man of kindly and reserved temperament, his loss is felt keenly by his many scientific colleagues and his acquaintances in the agricultural

industry. Plant Pathology has lost a promising recruit in a man who held defense of his country as his first duty. His record remains as an inspiration, alike to those who knew him and to younger men who follow him into this field.

Research papers in which Jolivette was an author are as follows:

- Internal black spot of canning beets and its control. *Canning Age* **19**: 489-491; 508. 1938. (With J. C. WALKER and J. G. McLEAN.)
- Studies on boron deficiency in garden beet (*Beta vulgaris*). (Abstr.) *Phytopath.* **31**: 23. 1941. (With J. C. WALKER.)
- The boron deficiency disease in cabbage. *Jour. Agr. Res. [U.S.]* **62**: 573-587. 1941. (With J. C. WALKER and JOHN G. McLEAN.)
- Boron deficiency in garden and sugar beet. *Jour. Agr. Res. [U.S.]* **66**: 97-123. 1943. (With J. C. WALKER and JOHN G. McLEAN.)
- Effect of boron deficiency on the histology of garden beet and cabbage. *Jour. Agr. Res. [U.S.]* **66**: 167-182. 1943. (With J. C. WALKER.)
- Productivity of mosaic resistant Refugee beans. *Phytopath.* **33**: 778-788. 1943. (With J. C. WALKER.)
- The limitations of spraying tomatoes in Wisconsin. *Wis. Agr. Exp. Stat. Res. Bull.* 152. 1944. (With J. C. WALKER, O. C. WHIPPLE, and W. J. HOOKER.)
- Varietal susceptibility in garden beet to boron deficiency. *Soil Science* **59**: 461-464. 1945. (With J. C. WALKER and W. W. HARE.)
- Yellows resistant cabbage varieties in the early and mid-season round head groups. *U. S. Dept. Agr. Tech. Bull.* (In press.)

SOME FEATURES OF THE SPREAD OF PLANT DISEASES BY AIR-BORNE AND INSECT-BORNE INOCULUM

E. E. WILSON AND G. A. BAKER¹

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Visual evidence of the spread of phytopathogenic fungi by wind is afforded in the development of the diseases on above-ground parts of susceptible plants near a source of inoculum. Plant pathologists have frequently reported the disease zones or gradients that appear in such location, but few have given the phenomena critical attention. They agree, however, that the decrease in the incidence of infection as distance from the source of inoculum increases is probably associated with a corresponding decrease in the density or frequency of the inoculum in the air during the dissemination period or periods. If this is true, it follows that the characteristics of the disease gradients might reflect the pattern of spore dispersion,² but since the disease results from the interplay of a large number of factors, some affecting spore dissemination and others influencing infection and the development of symptoms, the infection pattern would probably not possess features identical in all respects with those of the aerial spore pattern. According to Gregory (8), however, certain features of the gradients reported for various diseases are noticeably constant. This he believes is due in large measure to a fairly constant pattern of spore dissemination. Gregory (8), moreover, analyzes some of the published results on different disease and aerial spore density gradients, and shows a correspondence between these data and features pertaining to the transfer of atmospheric properties (heat, momentum, and suspended particles) by eddy diffusion.

The present writers (19) attempted to show the relationship between the spatial distribution of blossom blight, caused by *Sclerotinia laxa* Ader. and Ruh., in groups of apricot trees, and the pattern of spore dispersion; paying particular attention to effects of wind velocity on the pattern of spore dispersion. When a study of data collected by others was undertaken, however, it became apparent that the individual effects of the numerous factors involved could not be determined, because information regarding these factors was not given in the published reports. It was desirable, therefore, for the analyses of such data to follow a procedure that would provide for the effects of a large number of undetermined variables without being cumbersome.

The writers wish to describe a method based on the results of their (19) earlier studies of spore dispersion and to compare this method with that

¹ Associate Plant Pathologist and Assistant Statistician, respectively.

² The scattering of spores in the dimensions transverse to the main direction of the wind. Meteorologists employ the term "diffusion" to describe a similar process in the transfer of atmospheric properties by eddy movement.

described by Gregory (8). Inasmuch as the mathematical procedures involved are more or less standard, they are not described in detail.

CORRELATING THE PATTERN OF DISEASE INFECTION WITH THE PATTERN OF SPORE DISSEMINATION

Seven gradients of brown-rot blossom blight were reported by the writers (19); 3 for 1939 and 4 for 1940. Though the rate of decrease of infection with distance was greater in 1939 than in 1940, the curves representing the incidence of blossom infection in trees receiving the inoculum, when expressed as percentages of the incidence in source trees, were described by the equation:

$$y = \frac{A}{x^p} \quad (1)$$

where y was the incidence in recipient trees, x the distance in feet from the center of the nearest source trees, and A and p were constants depending on the wind velocity during the period of "effective dissemination,"³ and perhaps on other quantities to a lesser extent.

According to results obtained by releasing spores and intercepting them at different distances downwind, the aerial density,⁴ y , of these bodies diminished about inversely proportional to the square of the distance from the source. Hence, if the density at the station, x , nearest the source at which spores were intercepted was considered to be 100, the densities at subsequent distances, expressed as percentages of the density at x_1 , were described by

$$y = \frac{100}{x^2} \quad (2)$$

According to other tests (19), moreover, the dispersion of air-borne spores, over open ground at least, increased approximately proportional to the distance from the source. Thus, the chances for infection of recipient plants downwind from the source of spores would probably diminish inversely proportional to some power of x , the distance from the source.

In the studies (19) of apricot blossom blight it was possible to determine the incidence of infection at the source. With many diseases, however, this is frequently impossible, since the source may be an alternate host of the fungus, a group of plants differing in susceptibility from the recipient plants, or in some instances refuse such as piles of potato tubers (2). In order to compare the rate at which infection diminishes with distance, therefore, it is necessary to take as a reference point the incidence of infection at the location nearest the source at which observations are made. This was the procedure adopted for studying aerial spore densities. In these spore dissemination studies the distances from the source (point of release) were $x_1 = 5$, $x_2 = 10$, and $x_3 = 15$ feet. As it happened, the density values obtained at these distances led to the relationship expressed by

³ Dissemination resulting in the initiation of infection in the recipient hosts.

⁴ The number of spores passing through unit area of a plane transverse to the mean direction of the wind.

equation 2. Suppose, however, that instead of intercepting the spores at 5, 10, and 15 feet, they were caught at 10, 20, and 30 feet. In the first case the spore density values at 10 and 15 feet would be expressed as percentages of the value obtained at 5 feet, and in the second case the values at 20 and 30 feet would be expressed as percentages of the density obtained at 10 feet. In both cases, however, the relative distances would be the same,

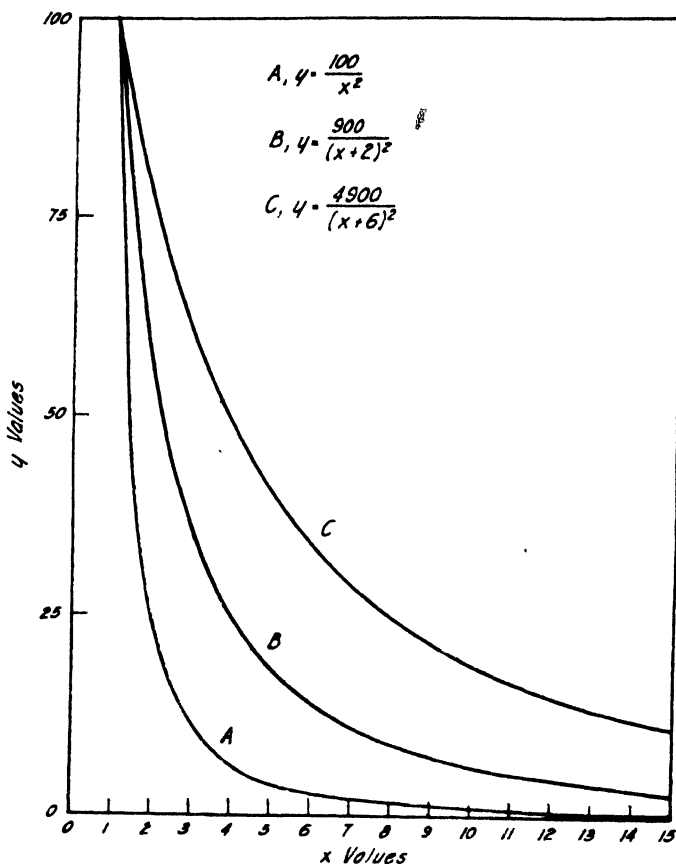


FIG. 1 Curves expressing the density, y , of air-borne spores at increasing distances, x , from the source of spores. The unit in which x is measured is the distance from the source to the nearest station, x_1 , at which observations are made. The critical distance from the source is such that if x_1 is at this distance the observed data will follow A, the inverse square law. Increasing x_1 by two or six times the critical distance, on the other hand, produces curves B and C respectively.

1, 2, and 3, etc. If, as indicated by experiments (19), the density values obtained at 5, 10, 15, etc., feet from the source were expressed by equation 2, the results would plot curve A, figure 1. Judging from this curve, the rate at which the values approach the x axis diminishes rapidly as absolute distance from the origin increases. If, therefore, spore density was measured at 10, 20, and 30 feet, the slope of the gradient would be less than that represented by curve A. Since the same relative distances are

involved, the curve would originate at $x = 1$, but for successive values of x it would approach the x axis less rapidly than A. In order to adjust for the effects of varying the absolute distance from the source to the first observation station, the quantity a is introduced into equation 2 as follows:

$$y = \frac{100 (1 + a)^2}{(x + a)^2}. \quad (3)$$

To determine the curve of best fit for results which vary with respect to the location of x , some rule for selecting a must be found. Equation 3 contains only one constant, a , to be determined, so we are to impose a single condition, the simplest and most desirable of which, perhaps, is that the sum of the differences between the observed and theoretical values be zero. The method for calculating a is a standard mathematical procedure.

To illustrate, the slopes of curves produced by equation 3, curves A, B, and C in figure 1 are plotted for a values of 0, 2, and 6 respectively. Increases in the value of a decrease the slopes of the curves.

In as much as the original data and details regarding the circumstances under which results were secured can be obtained from the literature cited, the presentation herein is confined to the percentage relationships between disease development at various relative distances from the source of inoculum. These percentages are computed from the original data, which in some cases were tabulated but in others appeared in figures and maps.

DISEASE GRADIENTS RESULTING FROM AIR-BORNE INOCULUM

According to Stepanov (17), both Gorlenko and Grushevoi determined the spread of crown rust of oats in one direction from the alternate host, *Rhamnus* sp. Grushevoi's results are expressed as the average "grade" of infection in an area "immediately neighboring" the source and at 10 and 25 "sazhen" (70 and 175 feet) from the source. Since this leaves only two points on which to establish a curve, Grushevoi's data are of little value. In figure 2, however, the curve is drawn on the assumption that the first point was 5 "sazhen" from the source. The distance at which Gorlenko made his first observations is not given, so computations are based on infection at the second distance, 45 meters.

Through the kindness of Dr. C. A. Suneson of the Division of Cereal Crops and Diseases, U. S. Department of Agriculture, the senior writer observed in the spring of 1945 a case where crown rust spread from groups of wild oat plants growing along an irrigation ditch running east and west. These plants, having obtained moisture from the ditch in late summer and early autumn, were much earlier to develop than other oats in the vicinity. They were infected by *Puccinia coronata*, probably in the autumn of 1944 before oats in the vicinity started development. In any event, a marked gradient of new infection by the crown rust fungus extended from these plants northward through the stand of less mature plants. In April the senior writer secured one set of counts on the number of lesions per 100 sq. cm. of leaf surface in recipient plants at different distances from the

edge of a group of source plants extending for a distance of 3 feet along the ditch bank. Unfortunately, further counts were precluded because lesions resulting from secondary infection appeared shortly thereafter. The results, together with the curve of best fit, appear in figure 2.

Although the number of points at which the Russian workers made their observations are few, and the first point in Grushevoi's data is an assumed value, curves are fitted to them very satisfactorily by equation 3. The senior writer's results also are fitted satisfactorily. Here the number of

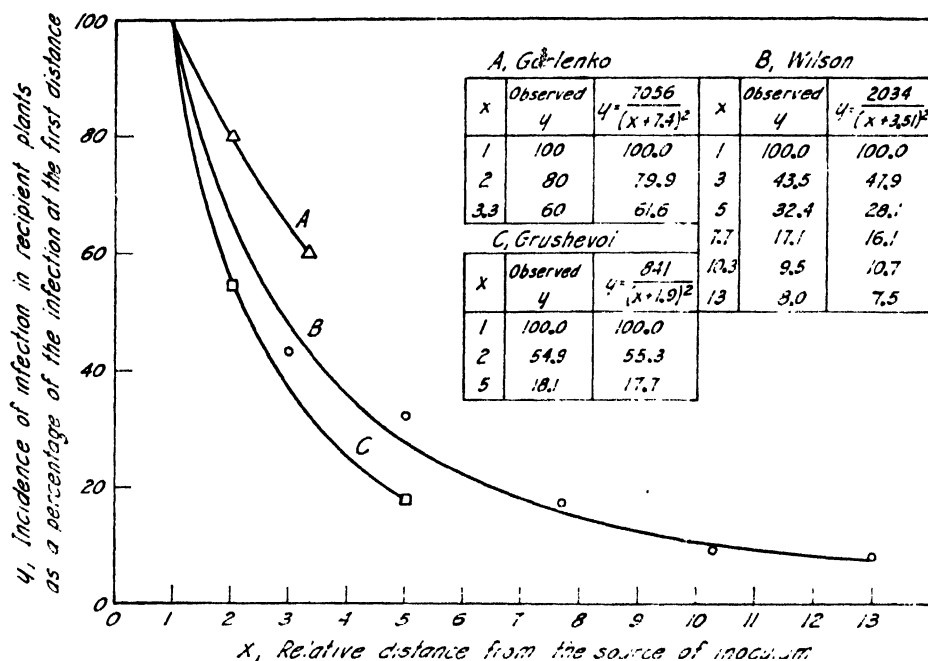


FIG. 2. Gradients of crown rust infection on oat plants in the vicinity of buckthorn bushes (Gorlenko and Grushevoi) and on young wild oats in the vicinity of more mature wild oat plants which served as a source of spores (Wilson).

observation points were more numerous and, consequently, the trend of the data appears in greater detail.

Results reported by Buchanan and Kimmey (3) and Posey and Ford (15) pertaining to the spread of the blister rust from currant bushes to pine trees are represented fairly well by equation 3 (Fig. 3), the major difference between observed and computed results being in the tendency for the slope of the gradient at two or three points near its outer boundary to be greater than that of the curve. For example, in curve A from relative distances 1 to 11 the trend of the observed percentages is reasonably well represented by the curve, but for relative distances 13 to 17 the observed values decrease more rapidly than the curve. Similar tendencies were noticeable in the gradient north and south of the source, and in gradients of a few other diseases. Though one might conclude that such

data might be more exactly represented by an equation in which the exponent of x was greater than 2, the rapid decrease in the value obtained at the outer boundary of a gradient is possibly due to chance events such as the nonuniform distribution or grouping of the recipient hosts, or to observations on insufficient numbers of individuals.

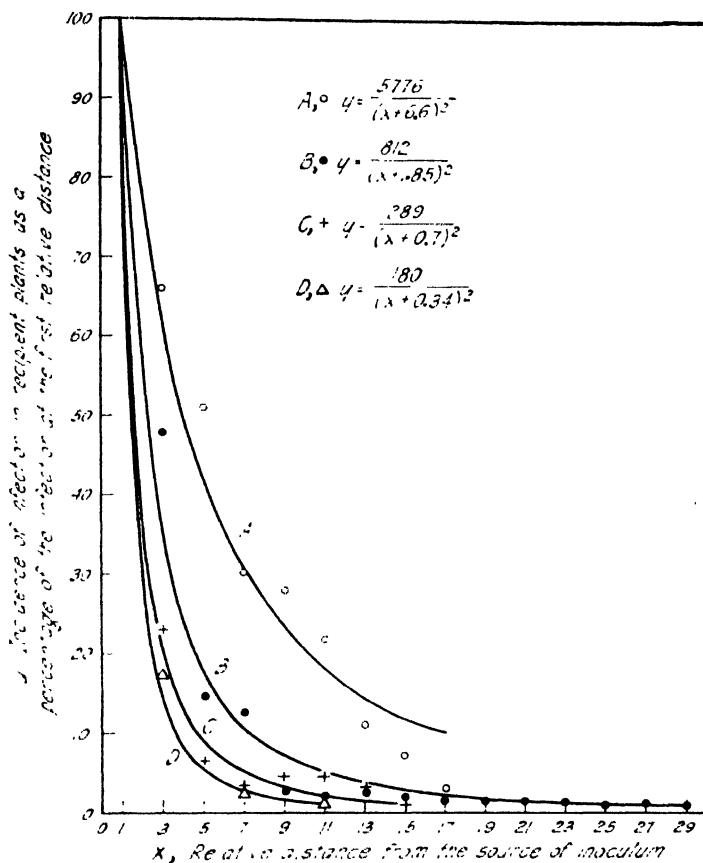


FIG. 3. A and C, gradients of blister rust infection in pine trees, west and east, respectively, of a group of currant bushes (Posey and Ford). B, a gradient of blister rust infection in pine trees surrounding a group of currant bushes (Buchanan and Kimmey). D, a gradient of late blight infection in a potato field near a pile of refuse potatoes.

Bonde and Schultz (2) observed the spread of late blight, caused by *Phytophthora infestans* from refuse piles of potato tubers to nearby potato fields. Table 3 of their paper reports the percentage of recipient plants infected at various distances from the source and the number of lesions per 100 plants at these distances for one field. While the percentage of plants infected cannot be represented by equation 3, the data on the number of lesions is roughly fitted. On the other hand, the average percentages of plants infected in five other fields are well represented by equation 3, as can be seen in curve D, figure 3.

The foregoing data were selected as fair representations of the apparent general agreement between observed results and the concept that a basic pattern is reflected in most of the disease gradients. Further indications of this are seen in Boevsky's (1) studies of the spread of leaf rust of wheat (results for June 13). Here the percentage infection at 50, 100, and 200 meters from the source corresponds very closely to equation 3 with an a value of 4. Results on the spread of tulip spot given by Wallace (18) and compiled by Gregory (8, table 13, observations for April 4) are in good

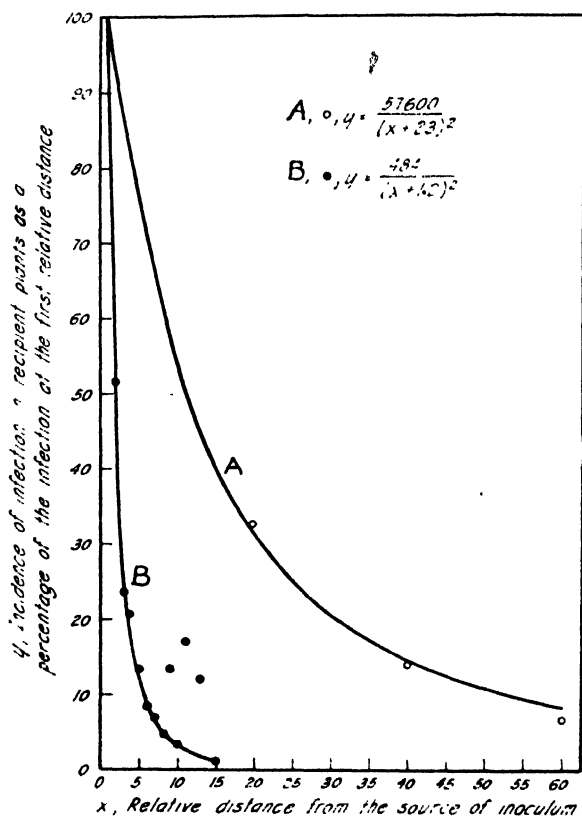


FIG. 4. A, a gradient of yellow dwarf infection in a potato field adjacent to a meadow from which the insect vector migrated (Frampton, Linn, and Hansing). B, a gradient of angular leaf spot infection in cotton plants east of a row inoculated with the causal bacterium (Faulwetter).

agreement with equation 3, having an a value of 0.76. Two out of three gradients of loose smut of wheat reported by Oort (13) are described fairly well by equation 3 with a values of 2.84 and 6.5.

In certain cases reported by other workers the percentage infection at successive distances decreases irregularly, and in consequence, no type of smooth curve can be made to pass through more than a few of the observed values. By and large, however, the general trend of the data is expressed by equation 3 in the following cases: cedar-apple rust (16), downy mildew

of onion (11), powdery mildew of barley (14), and eye spot of wheat (12). On the other hand, the spread of black stem rust of wheat from barberry to wheat reported by Johnson and Dickson (9) is not represented by equation 3.

According to Faulwetter (4), the bacterium, *Phylomonas malvacearum*, causing angular leaf spot of cotton is spread in wind-blown droplets of rainwater spattered from the surface of infected leaves. Faulwetter (5) presents maps illustrating the spread of this disease from a row of inoculated plants in the middle of a cotton field. From Faulwetter's figure 1 the present writers compiled data pertaining to the number of lesions per plant in 14 successive rows east of the portion of the row inoculated with cultures 140, 143, 148, and 151, and in 4 successive rows east of the portion inoculated with cultures 141, 146, 149, and 153. Heavy infection of a few plants in rows 9, 11, 12, and 13 raised the averages for these rows. The location of these plants in groups suggested the possibility of natural infection having occurred on certain plants in these areas and a subsequent spread to surrounding plants. When the high values in these four rows were included in the calculations of the a value, the resulting curve followed the trend of the majority of points but passed above the lower values. Omitting the high values from computations, however, resulted in a satisfactory fit (lower curve, figure 4), the a value being 1.2. Data from the four rows east of cultures 141, 146, 149, and 153 were described very closely by an equation in which $a = 2.01$.

GRADIENTS FOR DISEASES SPREAD BY INSECT-BORNE INOCULUM

At first thought the spread of plant disease by means of an insect vector would appear to have little in common with the spread of disease by air-borne spores. The individual insect flight or hop would seem to be so erratic as to preclude a sustained spread in any one direction. In cases where the insects develop on one crop and, obtaining the pathogen on this crop, migrate to another, however, there is an expansion, in some cases unilaterally, of the area in which the insect occurs. According to studies by Linn (10) and Frampton, Linn, and Hansing (6), the migration of viruliferous leafhoppers from source or reservoir plants into adjacent plantings of susceptible crops, results in distinct gradients of infection. Linn (10) noted that the percentage of yellows in successive rows of endive plants in the vicinity of weeds from which leafhoppers had migrated "was in inverse ratio" to the distance of the row of endive from the weeds. Frampton, Linn, and Hansing (6) compare the spread of these insects across the boundary between two crop areas to the flow of a compressible fluid and the components of this spread—the average length of insect hop—to the mean free path of gas molecules. It might be said here that such a concept is similar to that regarding the movement of atmospheric "turbulence bodies" or eddies which are responsible for the dispersion of air-borne objects (7). In developing an equation to express the spread of virus

diseases of the yellows type, Frampton, Linn, and Hansing (6) employ the concept of an unilateral diffusion process.

It is not the purpose to examine their treatment here, but by means of equation 3 to compare the gradients of diseases arising from the insect-spread of the inoculum with those arising from the aerial spread of the inoculum.

In his figure 9 Linn (10) plotted the percentage of yellows in rows of endive plants at increasing distances from a weedy strip from which the leafhoppers migrated. The results given in his curves A, C, and D were converted into the form suitable for application of equation 3. The trend of the results in curve B was too uncertain for a profitable treatment.

The results from curves A and D follow equation 3 to a noticeable degree, the values of a differing but little. In curve C, however, the data do not follow this relationship but seemingly are best described by an equation for a straight line.

Frampton, Linn, and Hansing (6) illustrate (figure 1 of their paper) the manner in which yellow dwarf was spread from a meadow into a potato field by the clover leafhopper. For the first 60 potato rows the percentage of plants infected progressively decreases as distance from the meadow increased, but because of the entry of leafhoppers from an uncultivated strip on the side of the potato field opposite the meadow, the incidence of disease increased somewhat in subsequent rows. The data to be examined, therefore, are those of rows 1, 20, 40, and 60.

The exact distance of row 1 from the edge of the meadow is not given, although the width of the rows is said to be 18 inches. Consequently, one cannot compare infection as to relative distances as we have been doing. It is possible, however, to fit the data in relation to the incidence of disease in row 1, though a very high value for a results. When this is done, however, (Fig. 4), we see that equation 3 apparently describes the data satisfactorily.

Zentmyer, Wallace, and Horsfall (20) established plantings of young elm trees around isolated old trees which were infected by the Dutch elm disease and also were infested by the insect vector of this disease, *Scolytus multistriatus*. Two years later they determined the percentage of young trees attacked by the disease in concentric zones whose outer boundaries were respectively 25, 75, 175, and 320 feet from the source trees. The mean percentages of 3 such plots were taken from their table 1. There is only fair agreement between observed and calculated results because the percentage at the last relative distance drops very rapidly, an irregularity noted in other disease gradients.

COMPARISON OF EQUATION 3 WITH AN EQUATION FOR THE TRANSFER OF ATMOSPHERIC PROPERTIES BY EDDY DIFFUSION

The transfer of heat, momentum, and matter by eddy diffusion in relation to the dispersion of spores is discussed in a recent paper by Gregory

(8). A correspondence is shown between the pattern of spore dispersion and the characteristics of disease gradients on the one hand and the characteristics of eddy diffusion on the other hand. It is proposed to compare Gregory's treatment with that employed in the present paper. Gregory begins by assuming the effect of gravity on most air-borne spores to be small in comparison to the effects of forces which keep them afloat.⁵ Thus, clouds of air-borne spores may be regarded as suspensions in the atmosphere. Consequently, their dispersion from a source should follow a pattern similar to that followed in the dispersion of smoke by eddy diffusion. For a more detailed explanation of this procedure reference should be made to Gregory's (8) paper. Its essential features are as follows: Consider the emission of air-borne particles from a point at the origin of x, y, z coordinates (x being horizontal to the ground in the mean direction of the wind, y being horizontal to the ground and at right angles to the mean direction of the wind, and z being vertical to the ground). A quantity of spores is liberated as a large number of small clouds, which may or may not take the same direction, but which diffuse as they travel downwind. The standard deviation of the positions of the spores from their mean position (the x axis) at any given distance from the source is given by $\sigma^2 = \frac{1}{2} C^2 x^m$. According to Gregory (8), the gradient of spore deposition at all distances, x , from the point source will be described by:

$$d_w = \frac{p \ 2 \ Q}{\pi C^2 x^m}$$

where

d_w = mean deposition per sq. cm. straight downwind at distance x from the source.

p = a deposition coefficient or the proportion of spores that will be deposited out of the total number crossing 1 sq. cm. of surface.

Q = the total number of spores liberated.

C = a coefficient of diffusion regarded as constant by Gregory.

m = a number between 1 and 2 regarded as constant for any set of conditions and any particular time interval.

This equation describes diffusion from a point source. For diffusion from a line source at right angles to the wind, the treatment must be modified. However, the equation for a point source will apply if the distance from the source is more than four times the dimension of the line source (8, p. 42). Gradients for strip and block sources will be flatter than those for point or line sources. Such differences in the steepness of the gradient are determined by the exponent of x .

The quantity m requires some discussion. On page 41 Gregory says that m is an indicator of the degree of atmospheric turbulence and to a first approximation is independent of wind velocity. It is primarily affected only by those factors that tend to dampen or enhance turbulence. It has a value

⁵ Gregory presents evidence that the size of the spore, hence its rate of fall in still air, influences very little, if any, the proportion of spores reaching the ground.

of 1.24 for low turbulence and approaches 2 for extreme turbulence, a normal value being 1.75. Moreover, m increases with the length of the sampling periods. In case of dissemination periods extending over hours or days, therefore, the value of m is probably a maximum.

The quantity C is a coefficient of diffusion which is said to remain relatively constant for the layer of air near the ground. It is said to have a normal value of about 0.6.

According to the evidence in the foregoing section, equation 3 of the present paper expresses a number of disease gradients satisfactorily, but the question arises as to whether the eddy diffusion equation represents the situation more accurately. We shall first examine the results given by

$$d_w = \frac{p \ 2 \ Q}{\pi C^2 x^{1.75}} = \frac{p \ 2 \ Q}{1.13 x^{1.75}} \text{ and } y = \frac{100(1+a)^2}{(x+a)^2}$$

on comparable bases. To do this we assume that for the eddy diffusion equation the relative number of spores, $p \ 2 \ Q$, has a numerical value of 113, thus making $d_w = 100$ when $x = 1$. The equation becomes $d_w = \frac{113}{1.13 x^{1.75}}$.

Curve A in figure 5 represents the d_w values of this equation for thirteen relative distances from the source, whereas curve B is the plotted values of

$y = \frac{146}{(x+0.21)^2}$. The close correspondence of these curves is seen by inspection. The maximum difference between the ordinates of curves A and B occurs at $x = 7.76$, and is only 0.47 per cent.

We next consider the decrease in the slope of the eddy diffusion curve occurring when m diminishes to 1.24, during short sampling periods or when atmospheric turbulence is low. Curve C in figure 5 represents such a curve. Curve D gives the values of y for equation 3, in which $a = 1$. It is seen that the two curves correspond very closely near the source but diverge at distances farther from the source.

It is unlikely that such small differences as indicated between curves A and B will be revealed in disease infection data, or, for that matter, in data from studies of aerial density of spores. The slope of the eddy diffusion curve is slightly less than the slope of equation 3.

In general, the two procedures give very similar results so long as the value of m is 1.75 or higher. Flatter gradients are said to occur (10) when the source is a strip or a block. An examination of results given by Boevsky (1) and by Pape and Rademacher (14), regarding changes in disease gradients over a period of time, suggests that the steepness may be modified by other causes, one being secondary infection from spores produced on the recipient plants themselves. Secondary infection of course introduces complications which preclude adequate treatment by either of the two procedures.

No doubt many other factors modify the steepness of disease gradients. It should be noted that the constant a was originally introduced into equation 3 to adjust for variations in the distance of x_1 from the source. Appar-

ently, however, little or no correlation exists between this distance and the value of a . Presumably, therefore, the constant a depends upon other factors as well. According to the comparisons in the foregoing section, equation 3 appears to fit the trend of many observed data reasonably well. While the eddy diffusion equation may express the effects of meteorological factors somewhat more closely than equation 3, it does not cover the great differences in slopes exhibited by the observed results. For example, the

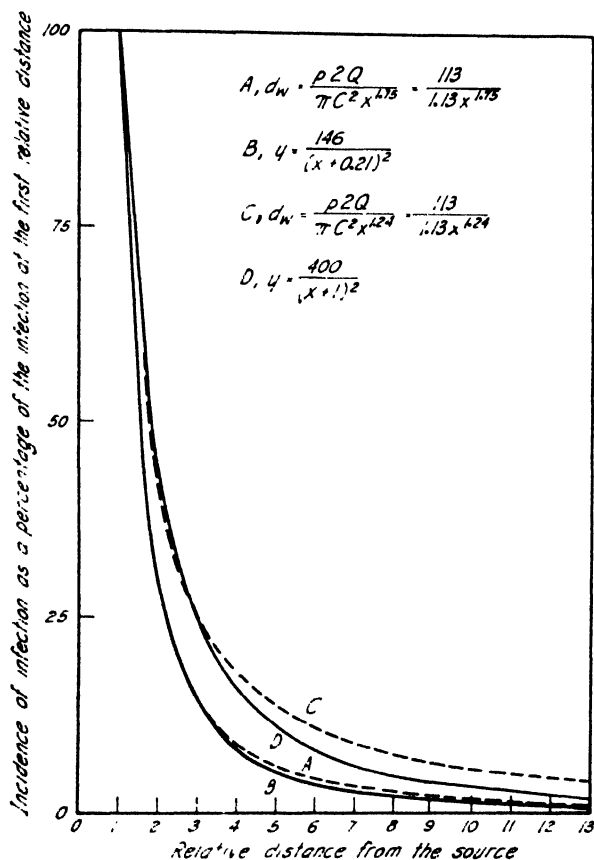


FIG. 5. A comparison of curves produced by the modified inverse-square equation with curves produced by Gregory's eddy diffusion equation having different values for the exponent of x .

minimum slope produced by the equation for a point source is illustrated by curve C, figure 5. On the other hand, many of the disease gradient curves are much flatter than curve C. The eddy diffusion equation, modified to represent dispersion from a block source, is scarcely applicable to certain of these gradients, since the sources cannot be considered blocks according to Gregory's (8) definition.

Hence, apparently the major difficulty in applying the eddy diffusion equation arises from its failure to account for the combined effects of the

large number of factors which affect characteristics of the gradients. The family of curves produced by equation 3, on the other hand, furnishes a much more flexible tool for at least a preliminary study of disease gradients. If and when the effects of particular factors are determined, such effects can be represented by appropriate constants, the values of which are then used to determine the value of a .

SUMMARY AND CONCLUSIONS

Numerical data pertaining to the spread of plant diseases from centers of infection to the above-ground parts of nearby susceptible hosts were compiled from the literature, and the rate at which the incidence of infection diminished with increases in distance from the source of inoculum examined. Some data collected by the present writers were also included. The diseases were of three categories with respect to the mode of inoculum dissemination: (1) Fungus diseases spread by means of air-borne spores. (2) A bacterial disease spread by means of wind-blown rain. (3) Virus and fungus diseases spread by insect vectors.

The gradients of infection that develop in the vicinity of sources of inoculum were compared with the pattern which in earlier studies appeared to characterize the aerial dissemination of spores. According to the results of these studies, for a distance of 15 feet at least from the source, the density, or number of air-borne spores passing through unit areas of planes perpendicular to the direction of the wind, diminished inversely proportional to the square of the distance from the source of inoculum, and the rate at which dispersion (the scattering of spores in dimensions perpendicular to the direction of the wind) increased was directly proportional to the distance from the source. Thus, the gradient of aerial spore density was described by the equation, $y = \frac{100}{x^2}$, where y is the density at x_2 and subsequent distance intervals expressed as a percentage of the density at x_1 , or first distance interval. In cases where the disease resulted from infection by air-borne spores, the decrease in incidence of infection at increased distance from the source was described to a fair degree of accuracy by a modified form of this equation, i.e.,

$$y = \frac{100(1+a)^2}{(x+a)^2} \quad (3)$$

Apparently the constant a in this equation depends upon the conditions under which spore dissemination occurs, as well as upon the location of x_1 with respect to distance from the source.

Data pertaining to the spread of angular leaf spot of cotton by wind-blown rain, yellows of endive by leafhoppers, yellow dwarf of potatoes by leafhoppers, and Dutch elm disease by bark beetles, suggest that in such cases, also, the relationship between incidence of infection and distance from the source, in some respects, is similar to that occurring in the spread of diseases by air-borne spores.

An equation, employed by Gregory for the transfer of atmospheric properties by eddy diffusion and representing the dispersion of air-borne particles downwind from a point source, was examined. When normal values for the constants of this equation were employed, the resulting curve differed little from the curve given by equation 3 when $a = 0.21$. At low atmospheric turbulence or during short periods of observing spore dissemination, the exponent of x in the eddy diffusion equation is said to be at its minimum value, 1.24. Under such conditions the curve of the equation represents a less rapid decrease in incidence of disease with increased distance than does the equation 3 curve which corresponds most closely to it at locations near the source. Thus, the two types of curves diverge at some distance from the source.

Field results, therefore, will probably not reveal the small difference in relationships between the two equations except possibly during times of very low atmospheric turbulence, or when the period of effective dissemination is very short.

The major difficulty encountered in applying the eddy diffusion equation to disease gradient data arises from its failure to account for the combined effects of a large number of variables. The family of curves produced by equation 3 seems more applicable to the diverse conditions met with in the field.

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REACTIONS OF OAT VARIETIES AND SELECTIONS TO FOUR RACES OF LOOSE SMUT¹

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Loose smut, *Ustilago avenae* (Pers.) Rostr., and covered smut, *U. kolleri* Wille (*U. levis* Magn.) are major diseases of oats in the United States causing an estimated average annual loss from 1935 to 1939 of 38 million bushels³ of grain.

These smuts may be controlled either by treating the seed, before planting, with New Improved Ceresan or formaldehyde, or by growing varieties highly resistant to smuts.

Breeding of oat varieties resistant to smut is complicated by the number of physiologic races of loose and covered smut that are known and being found. Sometimes these new or previously unknown races become prevalent after varieties resistant to known races have been bred and distributed for commercial production.

The purpose of the investigation reported here was to determine the reactions of a number of varieties and promising advanced hybrid selections of oats to 4 races of loose smut that have been collected recently in Kansas.

REVIEW OF LITERATURE

Reed (6) demonstrated 29 physiologic races of loose smut and 14 races of covered smut from collections of oat smut made in 15 states in the United States, 9 countries, and 4 continents. Navarro, Markton, and Victoria were inoculated with all of the races and were resistant in all cases.

Navarro and Victoria were highly resistant to races of smut in the United States as shown by results from the Cooperative Uniform Oat Smut Nurseries conducted in 9 to 15 states each year during 1935 to 1939 (13). Neither of these varieties had any smut during this period, which represents over 50 station-year tests for each variety. Markton was resistant, averaging 0.23 per cent smut for 65 station years. Because of its high resistance to smut and also to most races of crown rust, Victoria was used extensively as a parent for breeding varieties of oats resistant to smut and rust (5). Markton was used extensively also as a parent and Navarro also was used (2 and 5). A race of covered smut has been reported, however, to which

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³ U. S. Dept. Agr., Plant Disease Reporter. Supplement: 94, 1936; 100, 1937; 108, 1938; 118, 1939; 127, 1940.

Markton is intermediate in susceptibility. Smith and Bressman (10) inoculated seed of Markton, with hulls on and hulls off, with this race and obtained an average infection of 7 and 34 per cent smut, respectively. Unfortunately the collection of smut spores for inoculation by these investigators has been lost, thus precluding checking of any new races it may have contained.

In 1943, Reed and Stanton (7) demonstrated a new race of loose smut, collected in Oklahoma, to which Victoria and Fulghum were susceptible while Markton, Navarro, and Fultex were resistant. All of the 22 promising advanced selections of the cross Lee \times Victoria, which were resistant to all races of smut prior to this time, were moderately to highly susceptible to this new race.

MATERIAL AND METHODS

The 4 races of loose smut⁴ used in this study were collected in Kansas, although 2 of them were from fields planted with certified seed from other states. Race A was collected in a field of Kanota oats in Harper County in 1937 by J. O. Miller, Extension Plant Pathologist, Kansas State College. Fulghum and Monarch were susceptible to this race in 1940 after which the collection was propagated alternately on these 2 varieties. The inoculum used in 1945 was propagated on Monarch in 1944.

Race B was collected on an unknown variety of oats in Elk county in 1939 by J. O. Miller. Richland and Monarch Selection were susceptible to this race. The collection was propagated alternately on these two varieties. The inoculum used in 1945 was propagated on Monarch Selection in 1944.

Race C was collected by the writers in a test plot of Columbia oats in Sedgwick County, Kansas, in 1944. This plot had approximately 2 per cent of the panicles infected with smut. Certified seed of Columbia oats was obtained from a grower in Missouri for planting these plots.

Race D was collected in 1944 by the writers in a test plot of Fultex oats in Sedgwick County, Kansas, in the same field in which race C was collected. This plot had approximately one per cent of the panicles smutted. Certified seed of Fultex oats was obtained from a grower in Texas for planting these plots.

During the winter of 1944-45 the reactions of 9 smut-tester varieties and 18 other varieties to race D from Fultex were determined (Table 1). Seed with hulls off were inoculated with dry chlamydospores. Two replications of 125 seeds each of the 27 varieties were planted in a soil bed in the greenhouse. The temperature between planting and emergence was kept at 60° to 70° F. and the soil was maintained moderately dry. One hundred or more plants were obtained for each variety except for a few of the smut-tester varieties.

In the spring of 1945 the reactions to these 4 races of loose smut were studied on 12 smut-tester varieties, 21 named varieties, and 20 promising

⁴ These four physiologic races of loose smut will be tentatively designated as A, B, C, and D. Eventually they will be numbered according to a standard classification.

hybrid selections grown in the 1945 Cooperative Uniform Spring Sown Red Oat Test. In addition, all of the oat strains in the advanced yield test at Manhattan were tested to determine their reactions to race D. The seed with hulls on were inoculated by the partial vacuum method described by Zade (15) and modified by Haaring (3) using a suspension of 0.5 g. of chlamydospores in 100 cc. of water. The seed was dried and planted the following day in 2 replications in the field at the rate of 5 g. to an 8-foot

TABLE 1.—*Reactions of oat varieties to a race of Ustilago avenae that attacks Richland and Victoria varieties (Race D). Greenhouse, Manhattan, Kansas, 1944-45^a*

Variety	C.I. No.	Plants infected ^b
<i>Smut-tester varieties</i>		
Fulghum	708	3
Richland	787	90
Joanette	1762	100
Monarch	1876	100
Monarch Selection	1879	92
Gothland	1898	73
Victoria	2401	97
Red Rustproof	3212	0
Liberty Hull-less	845	100
<i>Other varieties</i>		
Brunker	2054	0
New Nortex	3422	0
Fulghum-Markton × Victoria-Richland	4140	0
Neosho	4141	0
Kanota	839	2
Navarro	966	2
Markton	2053	2
Bond	2733	2
Columbia	2820	12
Fulton	3327	26
Clinton	3971	26
Ventura	3989	53
Osage	3991	66
Boone	3305	91
Tama	3502	94
Vieland	3611	94
Cedar	3314	96
Fultex	3531	98

^a Seed with hulls off were inoculated by dusting chlamydospores on the seed.

^b Average of two replications, 4-foot rows.

row. Seed, with hulls off, of the same varieties were inoculated with dry chlamydospores of races C and D and then planted in the field in 2 replications at the rate of 2 g. to a 5-foot row.

The reactions of 8 smut-tester varieties, 18 named varieties, and 15 promising selections grown in the Cooperative Uniform Oat Smut Nursery were tested to a composite of several races of loose and covered smut which occur in Kansas, and to races A, C, and D. The composite did not include races A, B, C, and D nor the "Fulton" race of loose smut reported by Hansing *et al.* (4). Seed with hulls off were inoculated with dry chlamydospores

and then planted in the field in 2 replications at the rate of 1.5 g. to a 4-foot row.

In the field the soil temperature averaged 55° F. between planting and emergence. The soil was moderately dry the first 3 days after planting and then moderately moist for the following 4 days prior to emergence. Good stands were obtained for the group of varieties and hybrid selections planted with hulls on, while a fair stand was obtained for the rows planted with hulls off. A fair stand was obtained for some and a poor stand for others of the varieties and hybrid selections grown in the Cooperative Uniform Oat Smut Nursery.

In the greenhouse, percentages of infection were determined on the relative number of infected plants. In general, all of the panicles of a plant were smutted or all healthy. In the field, percentages of infection were determined on the relative number of infected panicles. Plants with some healthy panicles and some smutted panicles were common in the field.

EXPERIMENTAL RESULTS

Greenhouse Studies

Very high infection was obtained with race D in the greenhouse. Of the smut-tester varieties, Red Rustproof and Fulghum were resistant, averaging 0 and 3 per cent smutted plants, respectively (Table 1). The other smut-tester varieties were all susceptible, averaging from 73 to 100 per cent smutted plants. Victoria averaged 97 per cent smutted plants.

Brunker, New Nortex, Fulghum—Markton × Victoria—Richland, Neosho, Kanota, Navarro, Markton, and Bond were resistant, averaging from 0 to 2 per cent smutted plants. Columbia, Fulton, and Clinton were intermediate in susceptibility averaging from 12 to 26 per cent smutted plants. Ventura and Osage were susceptible, averaging 53 and 66 per cent smutted plants, respectively. Boone, Tama, Vieland, Cedar, and Fultex were very susceptible, averaging 91 to 98 per cent smutted plants.

Field Studies

Classification of physiologic races. Fulghum, Joannette, and Monarch were susceptible to race A, Gothland was intermediate in susceptibility, while all of the other 8 smut-tester varieties were resistant (Table 2). This race A represents a new race of loose smut distinct from any of Reed's 29 races (6). Reed did not have any race to which Fulghum and Monarch were susceptible while Canadian was resistant.

Richland, Canadian, Monarch Selection, Green Mountain, and Gothland were susceptible to race B, while the other differential varieties were resistant. This race B is similar to Reed's A-1 (6). It is commonly found where the variety Columbia is widely grown.

Richland, Canadian, Monarch, Monarch Selection, Green Mountain, and Gothland were susceptible to race C. Joannette was intermediate in susceptibility, while the other smut-tester varieties were resistant. This race C is somewhat similar to Reed's A-17.

TABLE 2.—*Reactions of oat varieties and selections to four races of Ustilago avenae in the field. Manhattan, Kansas, 1945*

Variety or selection	C.I. No.	Percentage infection by smuts ^a					
		Race A	Race B	Race C		Race D	
		Hulls on	Hulls on	Hulls on	Hulls off	Hulls on	Hulls off
<i>Smut-tester varieties</i>							
Fulghum	708	66	1	1	3	1	0
Richland	787	1	42	47	68	47	51
Canadian	1625	T ^b	90	88	98	92	98
Joanette	1762	50	0	17	22	78	92
Monarch	1876	82	0	62	77	92	98
Black Mesdag	1877	0	0	0	0	0	0
Monarch Selection	1879	T	86	78	84	80	84
Green Mountain	1892	0	82	78	92	75	94
Gothland	1898	12	86	68	78	11	26
Victoria	2401	0	0	T	0	85	88
Large Hull-less	3209	0	T	0	0	0	0
Red Rustproof	3212	0	0	0	0	0	0
<i>Named varieties</i>							
Markton	2053	T	0	0	0	0	0
Brunker	2054	0	T	0	0	0	0
New Nortex	3422	0	0	0	0	0	0
Neosho	4141	0	0	0	0	0	0
Navarro	966	0	0	0	0	T	0
Bond	2733	0	0	0	0	T	0
Kanota	839	69	2	2	4	T	1
Benton	3910	2	0	T	0	0	3
Clinton	3971	0	0	2	14	T	5
Marion	3247	0	3	1	3	T	6
Trojan	2491	0	6	5	16	3	6
Fulton	3327	0	3	4	4	4	5
Otoc	2886	0	2	42	46	2	9
Osage	3991	0	0	3	6	10	8
Ventura	3989	0	T	1	2	8	19
Columbia	2820	7	42	76	84	19	24
Boone	3305	0	0	0	0	69	79
Vicland	3611	0	0	0	0	72	84
Cedar	3314	0	0	0	0	74	83
Tama	3502	0	0	T	0	73	84
Fultex	3531	0	0	0	0	95	95
<i>Hybrids</i>							
Fulghum Markton × Victoria-Richland	4140	0	0	0	0	T	T
do	4251	0	0	T	0	0	0
do	4335	0	0	0	0	72	87
do	4528	0	0	0	0	T	0
Fulton × Victoria- Richland	4338	T	0	2	7	4	8
do	4339	0	0	3	3	5	20
do	4340	0	0	1	2	6	17
do	4524	0	0	T	T	4	26
do	4525	T	T	0	0	87	89
Columbia × Victoria- Richland	4153	T	2	1	4	1	1
do	4246	0	2	10	21	1	3
do	4344	0	5	2	8	0	0
do	4345	0	2	1	0	0	0

TABLE 2—(Continued)

Variety or selection	C.I. No.	Percentage infection by smuts ^a					
		Race A	Race B	Race C		Race D	
		Hulls on	Hulls on	Hulls on	Hulls off	Hulls on	Hulls off
do	4346	0	T	0	0	61	72
do	4347	0	0	0	0	52	63
do	4349	0	1	1	0	51	56
do	4527	0	1	12	30	1	2
Victoria-Richland ×							
Morota-Bond	4301	0	0	T	0	0	0
C.I. 4001 × C.I. 3644	4341	0	0	0	0	0	0
C.I. 4001 × C.I. 3842	4526	0	0	1	4	0	0

^a Seed with hulls on were inoculated by partial evacuation. Seed with hulls off were inoculated by dusting the chlamydospores on the seed. Percentages represent averages of 2 replications. Hulls on = 8-foot rows, hulls off = 5-foot rows.

^b T = 0.1 to 0.5 per cent infection.

Good infection was obtained with race D in the field for the highly susceptible varieties, although it was not so high as in the greenhouse. The infection for the varieties such as Ventura and Osage was not nearly so high in the field as in the greenhouse. Clinton and Fulton were intermediate in susceptibility in the greenhouse but had such low percentage of infection in the field that it was not possible to differentiate the relative susceptibility of similar varieties and hybrid selections from each other and from those which had high resistance.

Richland, Canadian, Joannette, Monarch, Monarch Selection, Green Mountain, and Victoria were susceptible to race D. Gothland was intermediate in susceptibility, while the other smut-tester varieties were resistant. The reactions of the smut-tester varieties demonstrate that race D is a new race of loose smut. It differs from the "Victoria" race which Reed and Stanton described as A-30 (7) in that Fulghum is resistant, while for their race Fulghum was susceptible.

In the Cooperative Uniform Oat Smut Nursery, similar infection was obtained for 8 smut-tester varieties to races A, C, and D (Table 3). For the Kansas composite of other races of loose and covered smut, Canadian, Monarch, and Fulghum were susceptible, with 93, 79, and 67 per cent smutted panicles, respectively. Gothland had 28 per cent smutted panicles, while the other smut-tester varieties had from 0 to 6 per cent smutted panicles.

Reactions of named varieties and hybrids. Kanota was susceptible to race A, with 69 per cent smutted panicles. All of the other varieties and advanced hybrid selections were resistant to this race, with from 0 to 7 per cent smutted panicles (Tables 2 and 3). Approximately 80 per cent of the oat strains had no smut.

Columbia was susceptible to race B, having 42 per cent of the panicles infected. The other oat varieties and hybrid selections inoculated with race

TABLE 3.—*Reaction of varieties and selections in Cooperative Uniform Oat Smut Nursery to a composite smut inoculum of loose and covered smuts and to physiologic races of loose smut in the field. Manhattan, Kansas, 1945*

Species, group, and variety	C.I. No.	Percentage infection by smuts ^a			
		Com- posite ^b	Race A	Race C	Race D
<i>Smut-tester varieties</i>					
<i>Avena sativa</i>					
Early black oats					
Monarch	1876	79	91	68	88
Black Mesdag	1877	6	0	0	0
Midseason white oats					
Canadian	1625	93	1	94	96
Gothland	1898	28	11	58	16
<i>Avena byzantina</i>					
Early red oats					
Fulghum	708	67	61	3	2
Columbia	2820	2	9	72	21
Midseason red oats					
Red Rustproof (Appler)	1815	0	0	0	0
Victoria	2401	0	0	0	90
<i>Resistant varieties</i>					
<i>Avena sativa</i>					
Early white oats					
Forvie	4164	0	0	0	26
Victoria Richland x Morota-Bond	4301	0	0	0	2
Early yellow oats					
Cedar	3314	0	0	0	82
Clinton	3971	0	0	4	2
Benton	3910	0	0	0	0
D69 x Bond	4259	0	0	2	1
Mindo	4328	0	0	0	0
Midseason white oats					
Mission	2588	0	0	9	3
Markton x Victory	2591	0	0	0	0
Markton x Ligowa	3025	0	0	0	0
Markton x Ligowa	3633	0	0	0	0
Bonda	4329	0	0	0	0
Victoria-Richland x Bannock	4181	0	0	0	0
Victory x (Victoria-Richland x Markton-Victory)	4523	0	0	0	0
Midseason yellow oats					
Markton	2053	0	0	0	0
Bond x Rainbow	4331	1	0	0	1
Bond x D67	4327	0	0	3	2
Goldwin	4237	0	0	0	1
Stanton Strain 2	4390	0	0	0	81
Florilee	4060	0	0	0	86
Lee Victoria x Fulwin	4316	0	0	0	92
Fulwin x Lee-Victoria	4383	0	0	8	
Traveler	4206	0	0	0	96
<i>Avena byzantina</i>					
Early red oats					
Ventura	3989	0	0	4	8
Osage	3991	0	0	2	9
Neosho	4141	0	0	0	0
Columbia x Columbia-Navarro	4241	0	0	15	0
Columbia x Victoria-Richland	4522	0	7	15	14
Columbia x Victoria-Richland	4346	0	0	0	26
Fulgrain Strain 7	4389	0	0	0	38
Victorgrain Strain 5	4388	0	0	0	70

TABLE 3—(Continued)

Species, group, and variety	C.I. No.	Percentage infection by smuts ^a			
		Com- posite ^b	Race A	Race C	Race D
Midseason red oats					
Bond	2733	0	0	2	0
Red Rustproof ^b × Victoria-Richland	4385	0	0	0	0

^a The hulls were removed before the seed were inoculated.

^b Composite of several races of *Ustilago avenae* and *U. kolleri* which occur in Kansas. *U. avenae* races A, B, C, D, and "Fulton" were not included in this composite.

B averaged from 0 to 6 per cent smutted panicles (Table 2). Approximately 60 per cent were free from smut.

Columbia was susceptible to race C, averaging 80 per cent infected panicles. Otoe, generally considered as a moderately resistant variety, averaged 44 per cent smutted panicles when inoculated with race C. Trojan, Mission, and Clinton averaged 10, 9, and 7 per cent smutted panicles, respectively, with this race. Four of the 10 hybrids which had Columbia as one parent were intermediate in susceptibility, averaging from 15 to 21 per cent smut (Tables 2 and 3). Apparently a recombination of genetic factors occurred in these crosses to give this intermediate type of susceptibility. The other varieties and hybrid selections averaged from 0 to 8 per cent smutted panicles. Approximately 50 per cent of the varieties and hybrids were free from smut.

Of the commercial varieties grown in the United States, Fulghum and Kanota were susceptible to race A, Richland and Columbia to race B, and Richland, Columbia, and Otoe to race C. However, many of the new varieties and promising hybrid selections were susceptible to race D. Fultex (Fig. 1, A), distributed in Texas in 1940 as a smut-resistant variety (11), averaged 95 per cent smutted panicles. Boone, Cedar, Tama (Fig. 1, B), and Vieland, distributed as smut-resistant varieties in one or more north central states from 1940 to 1943 (5, 9, 11, and 12), were all susceptible, averaging from 74 to 78 per cent smutted panicles. In contrast Fultex, Boone, Cedar, Tama, and Vieland have averaged from 0.0 to 0.3 per cent smutted panicles in the Cooperative Uniform Oat Smut Nurseries (13 and 14). Florilee, a selection from a Lee × Victoria cross and distributed in Florida in 1944 (14), had 86 per cent smutted panicles, while Traveler, distributed in Arkansas in 1944 (14), had 96 per cent smutted panicles. Stanton Strain 2, Fulgrain Strain 7, and Victorgrain Strain 5 had 81, 38, and 70 per cent smutted panicles, respectively. Ventura, distributed in California in 1943 (1), and Osage, distributed in Kansas in 1945 (1 and 4), were susceptible in the greenhouse but moderately resistant in the field, averaging 12 and 9 per cent smutted panicles, respectively. Columbia and Forvie were intermediate in susceptibility to this race.

Culms of Ventura and Osage with smutted panicles were approximately

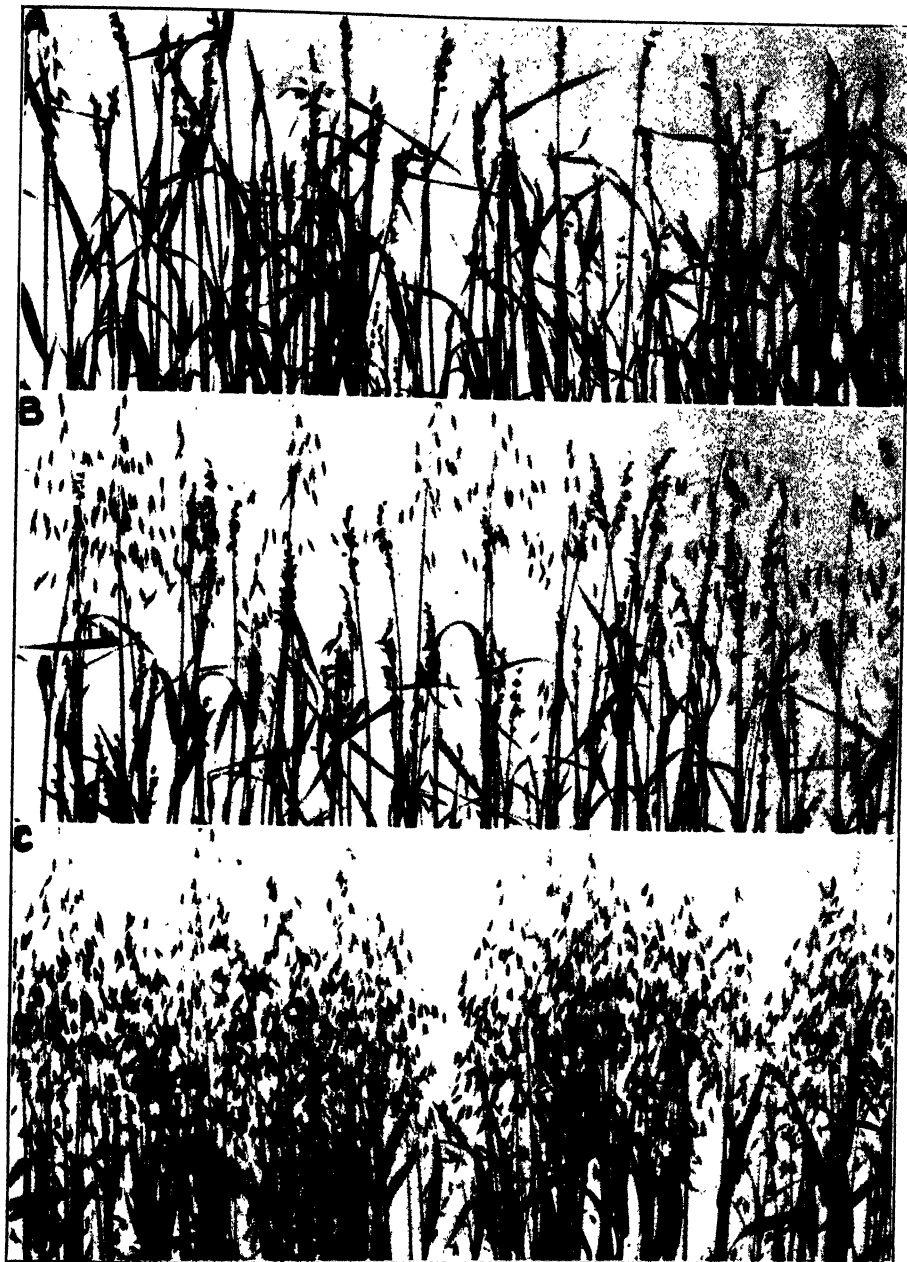


FIG. 1. Reaction of oat varieties to *Ustilago avenae* race D. A. Fultex, 95 per cent smutted panicles. B. Tama, 75 per cent smutted panicles. C. Neosho, 0 per cent smutted panicles.

40 per cent of the height of culms with healthy panicles. In contrast, culms of Boone, Cedar, Tama (Fig. 1, B), and Vieland with smutted panicles were approximately 90 per cent of the height of culms with healthy panicles.

Neosho (Fig. 1, C), distributed in Kansas in 1945 (1 and 4), was highly resistant. This variety has been highly resistant in other tests in Kansas and in the Cooperative Uniform Smut Nursery where it had an average of less than 0.1 per cent smut for 29 station years during 1943 and 1944 (14). It has yielded well in Kansas and in other states (1) and it is resistant to the common races of crown and stem rusts, but lacks resistance to stem rust races 8 and 10. Neosho is one of the few new commercial varieties with resistance to crown and stem rusts and as high or higher resistance to most races of smut as the old standard smut resistant varieties, Navarro, Markton, and Victoria.

Some of the hybrid selections were highly resistant while others were susceptible (Tables 2 and 3). Three selections from the cross Fulghum-Markton \times Victoria-Richland were highly resistant while one was susceptible. The resistant characteristic probably came from the Fulghum-Markton parent, since both of these varieties were resistant to race D, while the Victoria-Richland parent was susceptible.

In the cross Fulton \times Victoria-Richland, 4 selections were moderately resistant to intermediate in susceptibility to race D, having a reaction similar to Fulton, while one selection, C.I. 4525, was susceptible, averaging 88 per cent smutted panicles. C.I. 4525 or Kans. Sel. 41369 was highly resistant to the Fulton race of loose smut described by Hansing *et al.* (4). This would indicate that the reactions to race D and to the Fulton loose smut are probably determined by different genetic factors.

In the cross Columbia \times Victoria-Richland, 5 selections were found to have more resistance to race D than Columbia (Table 2). This resistance probably was due to recombination of genetic factors. Three selections were susceptible, similar to the Victoria \times Richland parent. When inoculated with race D approximately 30 per cent of the varieties and hybrid selections had no smutted panicles (Tables 2 and 3).

Out of 71 additional hybrid selections grown in the advanced yield nursery at Manhattan, 39 did not have any infection when inoculated with race D, 12 were resistant (trace to 10 per cent), 13 were intermediate in susceptibility (11-40 per cent), and 7 were susceptible (41-100 per cent).

DISCUSSION

The presence of a physiologic race of loose smut in the Southwest that attacks Richland and Victoria is of paramount importance because many of the new varieties of oats involving these strains as parents are widely distributed in the United States. As this race was introduced into Kansas on certified seed of Fultex oats from Texas, it may be assumed that it is already widely enough distributed that it can not be successfully eliminated by a seed treatment program. It may be only a matter of time until many of the new smut-resistant varieties now grown in the United States will be infected with this race so that the growers will have to treat their seed to control smut.

The occurrence of new physiologic races of oat smut is an important problem that confronts investigators who are breeding disease-resistant varieties of oats. In 1919 Kanota, a Fulghum type oat, was distributed in Kansas (1, 4, and 8) as a smut-resistant variety. It soon became a popular variety and its acreage increased rapidly. However, physiologic races of loose smut existed in the southern oat-growing area of the United States to which Kanota was susceptible. By 1928 these races of smut had spread in Kansas to such an extent that Kanota could no longer be considered as a resistant variety in the State (4).

In 1939, Fulton, a selection from a cross, Fulghum \times Markton, was distributed in Kansas (4 and 11) as a smut-resistant variety. Fulton was resistant to the prevalent races of smut in the United States, averaging 1.29 per cent infected panicles for 43 station-year tests in the Cooperative Uniform Oat Smut Nursery (13). However, with artificial inoculation it was intermediate in susceptibility to a new physiologic race of loose smut (4) that had existed as a slight mixture in a few of the Kansas collections of oat smut. This new race of smut was distributed with Fulton seed to growers of certified seed and by 1945 was distributed fairly well in Kansas. Fortunately Fulton oats was moderately resistant to this race of loose smut in the field. Since 1939, most of the fields of this variety have had none or only a trace of smut. However, as high as 10 per cent smut has been recorded in a few fields.

No smut was observed in the increase plots of Osage and Neosho in 1944. However, the seed was treated with New Improved Ceresan before it was distributed to growers of certified seed in 1945. It is desirable that growers of certified seed of these varieties continue to treat their seed.

Oat smuts are seed-borne diseases and are distributed principally on the seed. Growers of certified seed and other producers are partly responsible for the spread of new races of smut. It may be possible to slow down or even stop the distribution of a new race of smut if the seed of new varieties is treated properly to control smut. Another way of spreading new races of smut is through the interchange of new hybrid material among plant investigators in different states.

New varieties of oats that are resistant to known races of smut are bred by plant breeders and plant pathologists. Races of smut, however, exist or develop by hybridization or mutation some place in the country. These gradually spread until the new variety, after a decade or two, can no longer be considered a resistant variety.

In the meantime the plant breeder and plant pathologist may have a new variety ready for distribution, which is resistant to the old races of smut as well as to the new ones. In this way the research work on breeding disease-resistant varieties has generally managed to keep abreast of natural production of new races of smut. On the other hand, if seed dealers would treat all of their seed prior to selling, the problem of smut-resistant varieties becoming susceptible to new races of smut in the field

would be attacked from two angles. Progress in the development of new smut-resistant varieties of oats would probably keep farther ahead of the natural development and distribution of new races of smut than when attacked chiefly by the plant research workers alone.

SUMMARY

The four physiologic races reported in this investigation were collected in Kansas. Two of the races (A and D) were distinct from those reported by other investigators. Race A is characterized by the susceptibility of the varieties Fulghum and Monarch and the resistance of Canadian. Race D is characterized by the susceptibility of Richland and Victoria and the resistance of Fulghum.

Of the commercial varieties, Kanota and Fulghum were susceptible to race A. Richland and Columbia were susceptible to race B. Richland, Columbia, and Otoe were susceptible to race C.

Richland, Boone, Cedar, Tama, Fultex, Vieland, Florilec, and Traveler were susceptible to race D. Columbia and Forvie were intermediate in susceptibility. Ventura and Osage were susceptible in the greenhouse but intermediate to moderately resistant in the field. Benton, Clinton, Fulton, Mission, and Goldwin had some resistance to this new race of loose smut.

Black Mesdag, Large Hull-less, Red Rustproof, Navarro, Markton, Brunker, Bond, New Nortex, Neosho, Bonda, Mindo, and several promising hybrid selections were highly resistant to all 4 races.

Neosho appears promising as a parent for oat crosses. It is highly resistant to smut, resistant to the common races of crown and stem rusts and has desirable agronomic characters. Neosho was distributed in Kansas in 1945.

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SEED AND SEEDLING INFECTION OF BARLEY, BROMEGRASS, AND WHEAT BY *XANTHOMONAS TRANSLUCENS* VAR. *CEREALIS*¹

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Little is known concerning the seed and seedling infection of barley, bromegrass, and wheat by *Xanthomonas translucens* var. *cerealis*. Natural seed infection of barley and wheat has been reported, but no infection of bromegrass seed by this bacterium has been observed except for some lesions on the glumes enveloping the seeds (9). The seed-borne nature of *Xanthomonas translucens* on barley was described first by Jones, Johnson, and Reddy in 1916 (5). Seedling infection of barley from naturally infected seed was later described by Smith, Jones, and Reddy in 1917 (6), and the same process was postulated for wheat in 1919 (8). Jones, Johnson, and Reddy in 1917 (6) described barley seedling infection from barley seed naturally infected with *X. translucens*. In 1936, Bamberg (1) was unable to find a single infected wheat seedling in the field or in the greenhouse from seed naturally infected with *X. translucens*. In the same year, Hagborg (4) obtained 81 per cent infected seedlings from wounded wheat seeds which were infested with *X. translucens* and planted in the soil.

In 1941 Galachian (2) infected wheat seedlings with barley and wheat isolates of *Bacterium atrofaciens* by wounding the young plumules with a needle carrying the bacteria. In the same year Gorlenko (3) obtained 42 per cent diseased wheat seedlings by infesting wheat seeds with *Xanthomonas translucens* var. *cerealis* following an initial needle wounding of the seeds close to the embryo. Although Gorlenko and Hagborg showed that wounded infested seeds resulted in a high percentage of diseased seedlings they, and the earlier workers, did not define the course which the pathogen followed in moving from the seed surface to the aerial portions of the plant.

The purpose of this study was two-fold; first, to study the influences of races of *Xanthomonas translucens* var. *cerealis* on the growth of seeds and seedlings of barley, bromegrass, and wheat; and second, to trace the movement of this organism into the embryo and follow the spread of the pathogen from the seed up into the primary leaf.

MATERIALS AND METHODS

The barley and wheat varieties used were obtained from the Agronomy Farm at Iowa State College, while the strains of bromegrass were obtained

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from the Soil Conservation Service at Ames.³ As far as possible disease-free seeds were used. Prior to infestation all seeds were immersed for ten minutes in a 15 per cent solution of commercial clorox,⁴ and rinsed several times in distilled water.

The seeds were wounded by rupturing the testa covering the embryo with a dissecting needle, under a binocular. Four races of *Xanthomonas translucens* var. *cerealis*, whose history and performance were already known (9), were used because they were isolated from the same hosts employed in the present trials. All bacterial races were maintained on nutrient agar.

For seed infestation four 24-hour beef-peptone agar cultures of each race were used. The culture tubes were half filled with water and the bacteria were scraped into the water with a nichrome wire loop. The suspensions from 4 tubes of any one race were emptied into a single flask containing the seeds to be infested. The soaking seeds were then subjected to a partial vacuum by a water vacuum pump for 5 minutes and allowed to soak in the suspension for one hour or more depending upon the experiment. Usually the seeds were planted on sterilized moist filter papers in 9-cm. Petri dishes. In a few tests infested seeds were also planted in steamed compost soil in the greenhouse. The seedlings were usually examined for germination and disease seven days after plating in the laboratory.

THE INFLUENCE OF XANTHOMONAS TRANSLUCENS VAR. CEREA LIS
ON THE GERMINATION AND SEEDLING INFECTION OF
NONHULLED AND HULLED BARLEY SEED

While Hagborg (4) and Gorlenko (3) studied the pathogenicity of *Xanthomonas translucens* on wheat by infesting wounded seeds in bacterial suspension, Kingsolver (7) found that hulled oat seed without intentional wounding gave a higher percentage of infection than nonhulled oats when soaked in suspensions of *Pseudomonas coronafaciens*. This finding aroused speculation as to what might happen with barley and brome grass which are well fortified with hulls.

At the outset, the pathogenicity of races 1, 4, 5, and 6 of *Xanthomonas translucens* var. *cerealis* was tested on nonhulled barley in three experiments. No difference in germination and seedling infection was noted between the infested and the noninfested seeds. In further tests different lots of hulled and nonhulled Spartan barley were infested with race 6 by soaking in a bacterial suspension for one, two, three, and twenty-four hours. No differences in germination were noted in the hulled or nonhulled seeds, either infested or noninfested. The percentages of diseased seedlings in the infested nonhulled series were 12, 17, 17, and 50 for 1, 2, 3, and 24 hours' soak periods, respectively, while in the hulled series for the same periods the percentages were 54, 57, 57, and 67. The removal of the barley hulls had the

³ The author wishes to extend his gratitude to Dr. H. C. Murphy for supplying the seeds of the small grains. Also, it is a pleasure to acknowledge the assistance of Mr. M. L. Heath of the Soil Conservation Service at Ames in supplying the brome grass seeds.

⁴ Commercial clorox is a 1.5 per cent solution of sodium hypochlorite.

same effect on seedling infection as increasing the period of soak. In two additional experiments hulled and nonhulled Glabron barley were used. In the first of these the seeds were soaked for 1, 5, 10, 15, 20, and 25 hours, while in the second the seeds were soaked for 1, 5, 10, 24, and 58 hours. Again no influence on germination was noted for any of the treatments. In the infested series the percentage of infected seedlings was higher with the hulled seeds than with the nonhulled as in the earlier trials. With hulled seeds the number of infected seedlings was the same through the range of soaking periods. On the other hand, with the nonhulled seeds the number of diseased seedlings was greater with the longer soaking. In these trials the seedlings were infected without deliberate wounding of the seed. Of course, some wounding may have occurred in the process of removing the hulls.

THE INFLUENCE OF TEMPERATURE ON EMERGENCE AND SEEDLING INFECTION
FROM SEED SOAKED IN DIFFERENT STRAINS OF *XANTHOMONAS*
TRANSLUCENS VAR. *CEREALIS*

The influence of temperature on the pathogenicity of the organism on hulled and nonhulled seed was desirable to know. Also, with hulled seed it was essential to determine the influence of the temperature during germination on the seeds and seedlings. In a preliminary experiment with hulled barley infested with races 1 and 6 of *Xanthomonas translucens* var. *cerealis* and held at 15° C. and 25° C., no difference in germination was found and the percentages of infected seedlings were the same at both temperatures.

To determine whether or not the temperature during seed infestation influenced emergence and the seedling infection, nonhulled seeds of Glabron and Velvet barley were infested by soaking in suspensions of race 6 of *Xanthomonas translucens* var. *cerealis* for 24 hours at 10°, 15°, 20°, 25°, 30°, and 35° C. Corresponding lots of seed were soaked simultaneously at the different temperatures in distilled water. The seeds were then planted in potted steamed soil and were placed in a dark incubator at 25° C. When the young seedlings were 5 cm. high the emergence was recorded and the pots containing the seedlings were transferred to the greenhouse. Two days later the diseased seedlings were counted. (See table 1.)

The emergence of the two varieties was erratic in both infested and non-infested seed lots. At 30° and 35° C. a definite reduction of emergence was obtained in the infested and noninfested series. Seedling infection from the infested seeds occurred at all six temperatures. The total number of infected plants for all temperature exposures was 34 with Velvet and only 24 with Glabron barley, which is only 3 and 2 per cent, respectively, of the total number infested. Apparently during the period of soaking the temperature had no influence on the penetration of the bacteria beneath the hulls since the percentages of infected seedlings for both varieties were low at all temperatures.

The influence of temperature on emergence and seedling infection during the germination of seeds infested with races 1 and 6 of *Xanthomonas trans-*

TABLE 1.—*The emergence and seedling infection of two barley varieties soaked in water and in a suspension of race 6 of Xanthomonas translucens var. cerealis for 24 hours at six different temperatures*

Temperature, degrees C.	Velvet				Glabron			
	Water		Race 6		Water		Race 6	
	Emer. Pet. ^a	Inf. Pet. ^a	Emer. Pet.	Inf. Pet.	Emer. Pet.	Inf. Pet.	Emer. Pet.	Inf. Pet.
10	90	0	92	2	91	0	91	2
15	81	0	71	6	86	0	80	2
20	90	0	72	5	90	0	84	3
25	73	0	88	3	86	0	91	1
30	74	0	66	3	36	0	74	5
35	13	0	2	0	15	0	63	1

^a Each percentage represents the emergence from four replications of 50 seeds in each. Infection percentage was based on the number of seedlings that emerged.

lucens var. cerealis was studied. The seeds were infested by soaking them six hours at room temperature in suspensions of two races of the bacterium. The seeds were then planted, as before, in potted steamed soil and placed in the incubators until the seedlings were 5 to 7 cm. high, when the pots were transferred to the greenhouse. At this time emergence was recorded and seven days later seedling infection was recorded (Table 2). The emergence of the controls was at a high level, between 78 and 91 per cent, at temperatures of 10-25° C. and fell off slightly at 30-35° C. The emergence of the seeds infested with race 6 was lower than the controls at corresponding temperatures, except at 35° where there were no differences. The emergence percentage obtained using this race was the same at all temperatures except 30° C. where a reduction occurred. With race 1 the emergence decreased in a regular manner with increasing temperatures, from a high of 78 per cent at 10° C. to a minimum of about 12 per cent at 30° to 35° C. Therefore, there was less reduction of emergence in comparison to the controls at the lower than at the higher temperatures.

Seedling infection was obtained with both races of *Xanthomonas translucens var. cerealis*. The percentage of infected seedlings obtained with

TABLE 2.—*Emergence and seedling infection at six temperatures of hulled Glabron barley seeds infested with two races of Xanthomonas translucens var. cerealis*

Temperature, degrees C.	Water		Race 1		Race 6	
	Emer. Pet. ^a	Inf. Pet. ^a	Emer. Pet.	Inf. Pet.	Emer. Pet.	Inf. Pet.
10	89	0	78	6	60	13
15	78	0	65	12	60	10
20	91	0	50	22	63	16
25	78	0	31	16	58	14
30	67	0	12	33	47	15
35	61	0	13	77	63	14

^a Each emergence percentage represents the emergence from 4 replications with 25 seeds in each. The infection percentage is based on the number of seedlings that emerged.

race 6 was approximately 14 at all temperatures and no significant differences due to temperature were noted. With race 1 the percentage of infected seedlings was generally greater as the temperature at which the seedlings were grown was higher. Thus the percentage of infected seedlings ranged from a low of six at 10° C. to a high of 77 at 35° C.

THE RELATION OF SEED INJURY TO GERMINATION AND
SEEDLING INFECTION

The trials on nonhulled and hulled barley suggested that if the bacteria were allowed closer contact with the embryo they would penetrate the growing seedlings. Whether or not the pathogen entered the developing embryo was not known because the germinating seeds had not been killed. Hagborg (4) and Gorlenko (3) obtained high percentages of infected wheat seedlings by wounding and inoculating the seeds. These workers reported that wounding the seeds reduced the germination. In order to determine the influence of the bacteria on wounded seeds, trials were made with wounded barley, bromegrass, and wheat infested with different races of *Xanthomonas translucens* var. *cerealis*.

Barley Seed Wounded and Infested with Xanthomonas translucens
var. cerealis race 6

In the first trial two samples of Peatland barley of 20 seeds each were hulled and wounded; two other samples were hulled only. Similar lots of Spartan barley were also prepared. Using the two varieties, one sample of the wounded seed and one sample of the hulled seed were soaked in suspensions of *Xanthomonas translucens* var. *cerealis* race 6, while the other samples were soaked in water. The seeds were planted on moist filter paper in sterile Petri dishes. Germination was counted three days after planting, when the diseased plumules ceased to elongate. The short plumules were

TABLE 3.—Germination and seedling infection of two varieties of hulled barley artificially wounded and nonwounded soaked 20 hours in water and a suspension of race 6 of *Xanthomonas translucens* var. *cerealis* (20 seeds per plate).

Barley variety and trial number	Water				Race 6			
	Hulled		Hulled, wounded		Hulled		Hulled, wounded	
	Germ. Pet.	Inf. Pet. ^a	Germ. Pet.	Inf. Pet.	Germ. Pet.	Inf. Pet.	Germ. Pet.	Inf. Pet.
Peatland								
1	90	0	90	0	55	54	15	100
2	95	0	90	0	90	17	85	77
3	95	0	80	0	90	11	65	77
Spartan								
1	70	0	60	0	80	37	20	100
2	100	0	85	0	100	20	90	61
3	80	0	85	0	80	19	80	69

^a Percentage infection is based on the number of seedlings that developed.

frequently browned and appressed to the kernel. Final counts of diseased seedlings were recorded after seven days when the first foliage leaf had elongated sufficiently to have evidence of infection (See table 3).

In the control series the wounded and nonwounded hulled seeds of both varieties germinated well and the seedlings were healthy and vigorous. No significant reduction in germination attended the wounding of hulled seeds. In the series infested with bacteria the emergence of the nonwounded, hulled seeds was moderately reduced only in the first trial with Peatland barley, but not in the second or third trials. There was no reduction in germination with Spartan barley. With wounded hulled seed of both varieties there were marked reductions in emergence only in the first trial but generally none in the other two except for moderate reduction with Peatland barley in the third trial.

The percentage of diseased seedlings in all the trials of both varieties was much greater with the wounded, hulled seeds than with the nonwounded, hulled seeds. The occurrence of seedling infection in the nonwounded, hulled seed which was infested would suggest that wounding occurred during the hulling. An examination of hulled seeds through the binocular revealed that either the testa or the embryo were injured in many of the seeds.

Symptoms were observed on the young seedlings. The plumules that were not over 1.5 to 2 cm. long became yellow and water-soaked. On the coleoptile there were light brown lesions. Except for these inconspicuous lesions on the coleoptile some plumules appeared healthy. Later the lesions appeared on the first foliage leaf as it emerged from the coleoptile.

Bromegrass Seed Wounded and Infested with Xanthomonas translucens var. cerealis race 6

Preliminary experiments demonstrated that hulled bromegrass seed was more susceptible to *Xanthomonas translucens* var. *cerealis* than nonhulled seed. Therefore, three experiments were designed to determine if wounding increased the susceptibility of the germinating seeds to the bacteria. In another trial bromegrass seeds were hulled, divided into lots of 50 seeds, and wounded as before. The lots were then divided into two, 25 seeds each. One lot was soaked in water and the other was soaked in a suspension of the bromegrass pathogen. Twenty-five nonhulled and hulled seeds were included as checks against the hulled, wounded seeds. The germination and seedling infection were recorded five days following infestation. (See fig. 2.) The germination was the same in the nonhulled and hulled seeds in the controls. The germination of the hulled, wounded seed was reduced approximately 40 per cent, which indicated that the young embryos of the non-infested seeds may have been injured. Infestation reduced germination of nonwounded, hulled bromegrass seed and reduced it even more with wounded, hulled bromegrass seed. The lower germination in the nonhulled and hulled infested bromegrass seeds in comparison with the controls suggested that the bacteria had some influence on germination.

The infected seedlings were readily distinguished by the water-soaked lesions on the leaves. The percentages of infected seedlings based on the emergence were 4, 13, and 60 for the nonhulled, hulled, and hulled and wounded lots, respectively. There was no infection in the controls. Wounding the seed by splitting the testa favored the pathogen in its development in the seedling. Since the seedlings from nonhulled seed were infected, presumably the pathogen penetrated the hulls of the seed and contacted the embryo or the bacteria from the hulls contaminated the emerging coleoptiles.

*Wheat Seeds Wounded and Infested with Races 1, 4, 5, and 6 of
Xanthomonas translucens* var. *cerealis*

Wheat, which in preliminary trials had been unscathed by any of the test cultures, was wounded and soaked in suspensions of four races of *Xanthomonas translucens* var. *cerealis*. Nonwounded seeds were similarly treated. After soaking for 20 hours, the seeds were drained and planted in sterile Petri dishes or steamed soil. Control lots were soaked in water. Five trials were made at 2-day intervals, three in Petri dishes and two in steamed soil. Records were taken after seven days.

Germination was reduced in the wounded infested series. Races 1 and 6 caused the greatest reduction in germination. The embryos of some seeds were covered by a yellow bacterial mass. None of the four strains of bacteria seemed to attack the embryos of the nonwounded seeds and no differences were obtained in the percentage germination of the control and of the infested seeds.

As in the barley and bromegrass trials, the percentages of infected seedlings were greater with wounded than with nonwounded seeds. With bromegrass the difference between the two was very much greater than with the other crops. Race 6 was the least pathogenic. Races 4 and 6 produced less seedling infection than 1 and 5. In the soil the percentages of infection were 50, 40, 56, and 40 for the four races used. Seedling development was also influenced by the pathogen. For example, the average plumule length for the eight seedlings in the first trial of wounded seeds infested with race 5 was 2.6 cm., while the plumule length in the check was 7.0 cm.

THE RESPONSE OF NONWOUNDED SEEDS SOAKED IN SUSPENSIONS OF
XANTHOMONAS TRANSLUCENS VAR. *CEREALIS*
AND PLANTED IN SOIL

Laboratory trials with seeds soaked in a bacterial suspension showed that such infested seeds gave rise to infected seedlings in Petri-dish plantings. This raised the question as to what might happen when such infested seeds were planted in the soil. To study this problem an experiment was designed to determine the influence of races 1 and 6 on emergence and seedling disease with hulled and nonhulled barley seed. Lots of 200 seeds each were soaked in bacterial suspensions of races 1 and 6, and in water as control. After one hour of soaking under partial vacuum the seeds were planted, 40 seeds per

pot, in 4-inch pots of steamed soil. Five replicate pots for each of the twelve treatments were planted and randomized as complete blocks on the greenhouse bench. The emergence counts were made after seven days and are presented in figure 1.

An analysis of the data indicated a highly significant reduction in emergence of hulled seed due to bacterial seed infestation. No significant differences were noted between the two races. The emergence of nonhulled seed was unaffected by bacterial infestation. The hulls seemed to protect the seed from bacterial injury. With removal of the hulls, better emergence of the seed was obtained in the control series than when the hulls were left on.

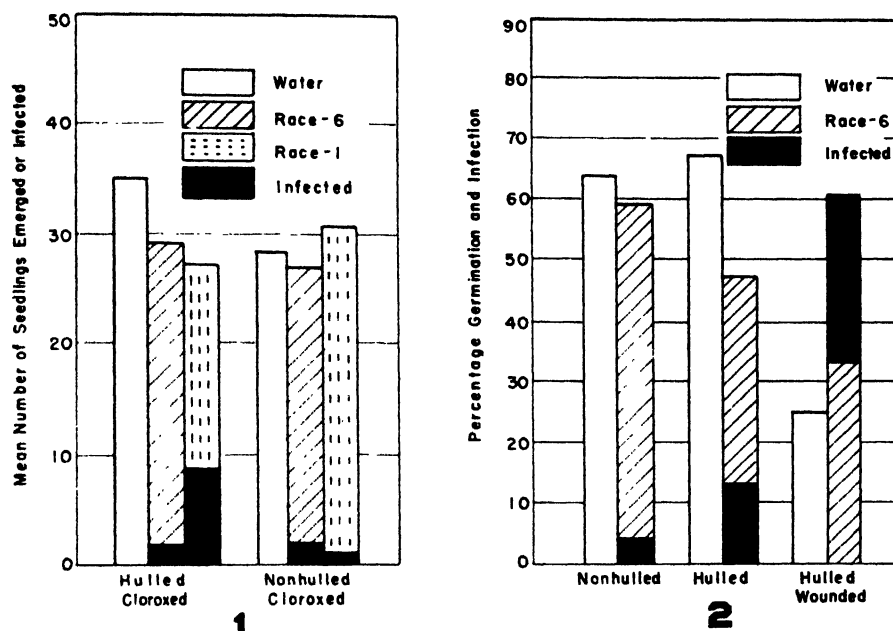


FIG. 1. The emergence and resulting number of infected seedlings which developed from hulled and nonhulled chloroxed barley seed soaked in suspensions of two races of *Xanthomonas translucens* var. *cercalis*. Seeds planted in steamed soil.

FIG. 2. The percentage germination and infected seedlings which developed from wounded and nonwounded bromegrass seeds soaked in a suspension of race 6 of *Xanthomonas translucens* var. *cercalis*.

The numbers of diseased plants recorded ten days following planting are in figure 1. The results indicated (1) seedling infection with both races of *Xanthomonas translucens* var. *cercalis*; (2) a greater number of plants infected with race 1 than with race 6; (3) a greater number of plants infected by both races when hulled seed was used. These infection results were in agreement with those obtained in the Petri-dish trials.

In a further experiment four races of the organism were compared. The nonhulled seeds were used and infested as before. The seeds were planted in potted steamed soil in four replications and randomized within a moist chamber where the temperature ranged from 15° C. at night to 25° C. dur-

ing the day. After seven days the pots were transferred from the chambers to the greenhouse bench and emergence was recorded. Seven days later notes were taken on the number of diseased seedlings.

A variance analysis of the data revealed a highly significant decrease in emergence due to the races 1 and 4, but not to races 5 and 6. All of these races induced low percentages of seedling infection, ranging from three with race 1 to six with race 5. Despite the low incidence of disease the experiment showed that infected seedlings could be obtained by planting infested seed in steamed soil in the greenhouse.

Wheat and brome grass seeds also were tested with the same four races. None of the races influenced emergence of these grasses although a few seedlings were diseased in each of them. All races caused some wheat seedling infection while only race 6 infected brome grass seedlings.

The small number of infected seedlings in these soil tests constituted further evidence that wounding favors infection. The data from the soil experiments substantiate the findings of the laboratory trials that wounding favors the entrance of the pathogen into the developing seedling.

THE MOVEMENT OF *XANTHOMONAS TRANSLUCENS* VAR. *CEREALIS* INTO THE SEED AND LEAVES OF THE DEVELOPING SEEDLING

Although Hagborg (4), Galachian (2), and Gorlenko (3) showed that diseased seedlings developed from wounded, infested wheat seeds, they did not attempt to explain how or where the bacteria entered the seed and the developing seedling. Two courses are open; either the pathogen contaminates the developing coleoptile as it emerges from beneath the hulls and subsequently passes through it to reach the first leaf or the pathogen penetrates beneath the hulls and testa of the seed and enters the young developing embryo. If the latter method is followed there must be a point in the embryo through which the bacteria would pass to reach the primary leaves of the seedling.

The present studies using nonhulled, hulled, and wounded hulled seeds have shown that high percentages of infected seedlings were obtained only when the seeds were wounded. When the testa over the embryo was split to allow for passage of the bacteria to the embryo, high percentages of the growing seedlings developed infected coleoptiles within 48 hours after infestation or had lesions on the first foliage leaves upon the leaf's emergence from the coleoptile. These facts indicated that the bacteria penetrated the coleoptile at some point soon after they were in contact with the tissues, since the symptoms were evident so soon after germination. Sections of diseased plumules present evidence to support this contention.

The symptoms on barley, brome grass, and wheat seedlings were remarkably alike. Many diseased seedlings, 48 to 72 hours old, were examined with the binocular. The first apparent symptoms on plumules 3-5 cm. long were yellowing, distortion, and deformation of the coleoptile. Long, water-soaked, sunken streaks were on some coleoptiles. These streaks which were apparent



FIG. 3. Diseased foliage leaf of a barley seedling with water-soaked, translucent areas. Note the bacterial exudate.

before the foliage leaf had burst through the coleoptile, became brown and later split, thereby exposing the yellow water-soaked inclosed foliage leaf. Severely diseased plumules soon collapsed and died, either before or after emergence from the coleoptile. In less severely infected plumules the first foliage leaves emerged from the coleoptile and continued to develop. Scattered translucent areas developed along the unfolded leaves and these areas later enlarged into yellow-green, water-soaked streaks (Fig. 3). These streaks were clearly visible on barley and wheat, but were difficult to see on the narrow leaves of brome grass. As the brome leaves increased in size, however, the streaks became clearly perceptible.

Free-hand sections cut from the base of the diseased plumule were studied under the microscope. Bacteria could be seen streaming out of the cut ends of the sections, but it was not always possible to determine the exact point of egression. Masses of bacteria and cell debris exuded from the tissues of the coleoptile and from the vicinity of the first foliage leaf. There were no lesions at the tip of the plumule nor was there any bacterial streaming from sections made at the tip.

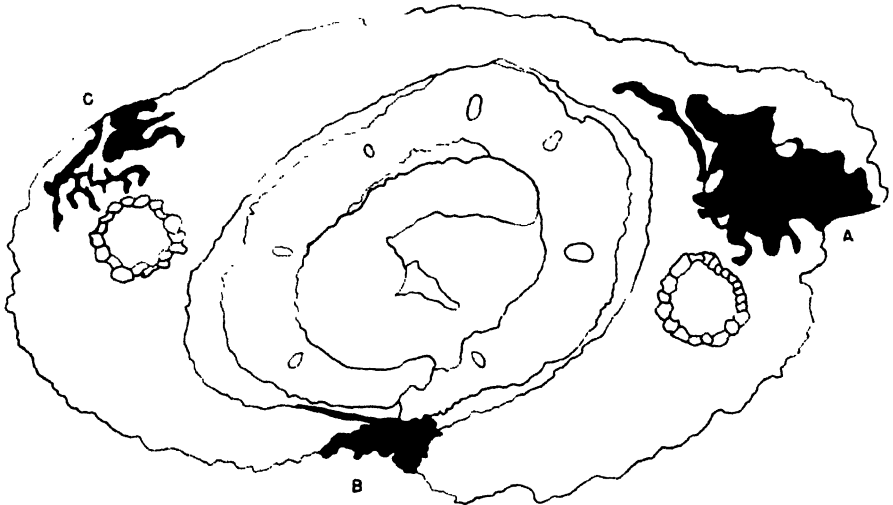


FIG. 4. A cross section of a barley plumule showing the lesions caused by race 6 of *Xanthomonas translucens* var. *cerealis*. The shaded areas indicate the points of entrance and the spread in the tissues. At A and C the bacteria were in the coleoptile while at B the parasite reached the first foliage leaf.

Plumules from which the free-hand sections had been cut in the preliminary diagnosis were killed in formalin-acetic-alcohol saturated with mercuric chloride. The mercury was added to prevent the leaching of the bacteria from the tissue. The tissues were run through the butyl alcohol series, embedded, and stained.

The sections were examined under the microscope for the location of lesions and the organism in the tissues. In these lesions there were masses of bacteria in the intercellular spaces extending from the outer to the inner surface of the coleoptile (Fig. 4). The first foliage leaf lying against the inner wall of the coleoptile was severely parasitized and partially disintegrated. The tip of the second foliage leaf abutting the first leaf was entirely destroyed and the space filled with debris and bacteria. The third leaf lying inside the second leaf was not invaded. The pathogen was intercellular in the parenchymatous tissues and it was never observed inside the endodermis.

There was no evidence that the bacteria invaded the seedling except through the coleoptile. Stomates were observed along the coleoptile and these served as natural openings for the entrance of the bacteria. Penetration was not observed in the root tissue or in the scutellum area. Primary infection presumably occurred through natural openings or wounds in the coleoptile either while in the embryo or in the early stages of plumule elongation. Presumably, the pathogen was carried up in the aerial parts of the seedling when the first foliage leaves emerged from the elongating infected plumules.

SUMMARY

Seed and seedling infection of barley, bromegrass, and wheat was studied with seeds infested with races of *Xanthomonas translucens* var. *cercalis*. The factors studied were duration of period during which the seeds were soaked in bacterial suspension, hulling and wounding the seeds, and the temperature during seed infestation and during seed germination and growth. The localization and the penetration of the bacteria into the developing plumule were studied also.

Hulled barley seed soaked for one hour in bacterial suspension produced 54 per cent infected seedlings while the nonhulled seeds which had been soaked in bacterial suspension for the same period produced only 12 per cent diseased seedlings. A 24-hour soak was necessary for the nonhulled seeds to yield 50 per cent diseased seedlings.

The temperature during seed infestation of nonhulled barley seed was not a factor influencing seed germination or seedling disease. The temperature during seed germination and seedling development influenced emergence and seedling infection when seeds were infested with race 1 of *Xanthomonas translucens* var. *cerealis*.

Infection of barley, bromegrass, and wheat seedlings was facilitated by rupturing the testa covering the embryo prior to seed infestation. Diseased seedlings were obtained from infested hulled barley and bromegrass seeds that were planted in the soil. A few diseased seedlings also developed from nonwounded infested wheat seeds.

Xanthomonas translucens var. *cerealis* penetrated through wounds in the pericarp covering the embryo, as demonstrated by the greater percentage of infected seedlings obtained from wounded seeds.

Infection of the plumule occurred through wounds or stomata on the coleoptile, spread rapidly through these tissues, and finally reached the inclosed foliage leaves. Since the first foliage leaf was adjacent to the coleoptile it became infected before emerging from the coleoptile. In this way, the infected leaf by elongation carried the bacteria into the aerial parts of the seedling. The initial symptoms were water-soaked streaks on the primary leaf.

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IDENTITY AND KNOWN DISTRIBUTION OF *ELSINOË PIRI* IN WASHINGTON AND OREGON

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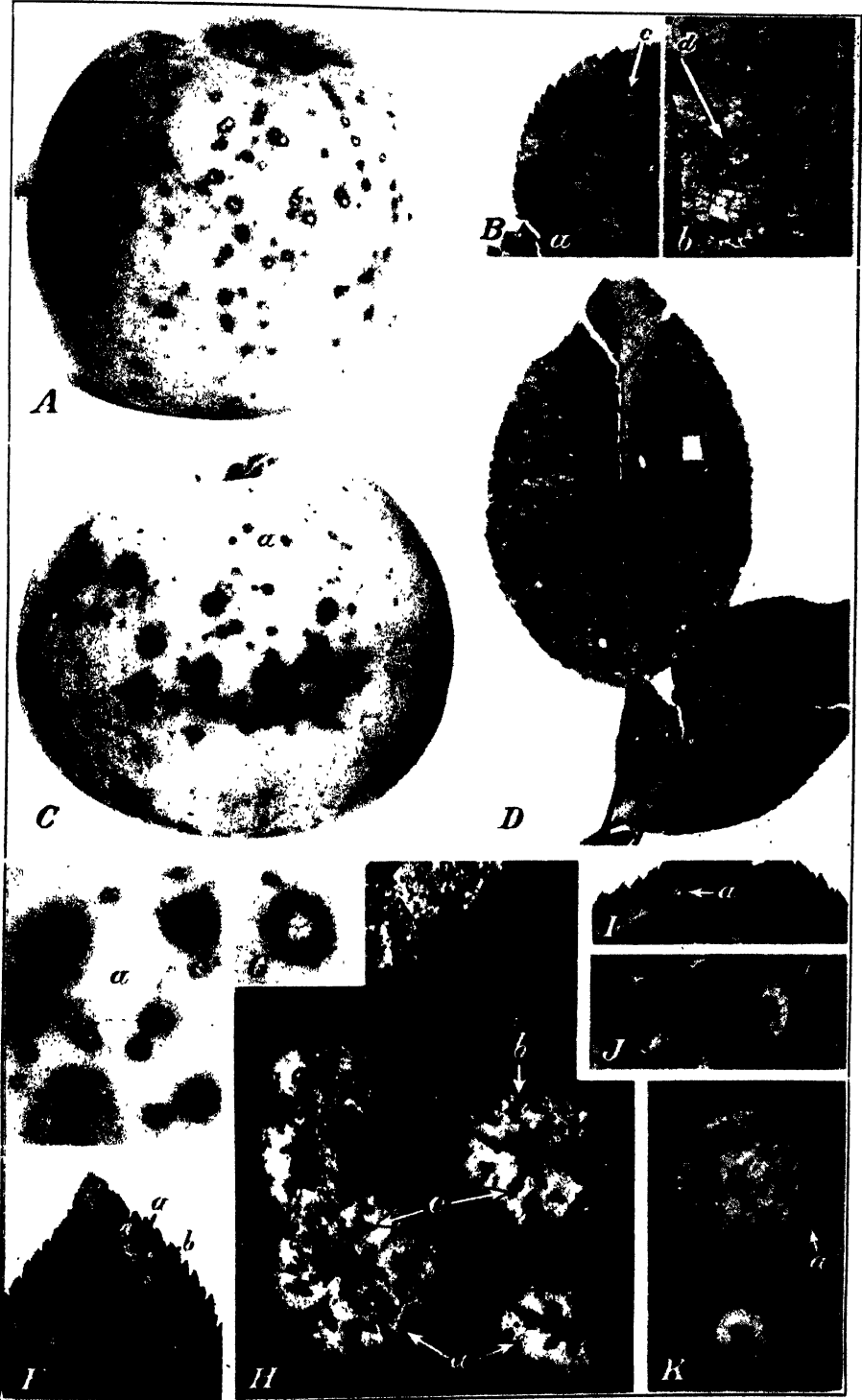
This paper presents certain basic data on the identity and known North American distribution of *Elsinoë piri* (Woronich.) Jenkins (3, p. 696) previously reported only in abstract form (2, p. 969 and 7). The recent findings in our Northwest of this pathogen,¹ hitherto reported only from Europe (3) and South America (3, p. 694; 5, p. 34-35; 6, p. 154), are of special moment in view of the economic importance of the susceptible fruit crop in the United States.

The first specimens examined were spotted apple leaves from Mt. Vernon, Skagit County, Washington, September 1, 1943, collected by M. J. Forsell and E. I. Smith, and spotted apple fruits from South Bend, Pacific County, Washington, October 10, 1943, collected by R. F. Wilbur, all three of whom participated in the Special Survey in the General Vicinity of Ports of Entry of the U. S. Bureau of Entomology and Plant Quarantine. These initial findings led to a special survey in 1944 in western Washington and Oregon by L. W. Boyle, of the Emergency Plant Disease Survey, U. S. Bureau of Plant Industry, Soils, and Agricultural Engineering, which was supplemented in Western Washington by M. J. Forsell.

Spotting typically produced by this pathogen is represented by the numerous specimens collected during September, October, and November over extensive sections of western Washington and Oregon. Examples of infected apple fruits and apple and pear leaves are shown in figure 1. Spots on the fruit of the Grimes Golden variety (Fig. 1, A) were generally "brick red"¹ (color reading based on dry apple peel) and pale at the center in the case of the older spots. Spotting was particularly colorful on a seedling apple fruit (Fig. 1, C). Smaller spots on this fresh apple were "pomegranate purple," larger more diffuse spots were "Eugenia red," and centers of older spots were purple brown. In contrast, the healthy apple skin in the

¹ Colors in quotation marks are based on Ridgway (10).

FIG. 1. Specimens of *Elsinoë piri* on apple and pear collected during the survey of 1944. A. Grimes Golden apple, Whatcom Co., Washington, autumn, M. J. Forsell. $\times 1$. B. Apple leaf, between Bellingham and Everson, Whatcom Co., Washington, September 21, L. W. Boyle; a, $\times 1$; b, part of a, showing dark conidial stage on the spots; d, same spot as c. $\times 3.5$. C. Fruit of an apple seedling, vic. Dayton, Yamhill Co., Oregon, Nov. 21, L. W. Boyle and J. A. Milbrath. $\times 1$. D. Pear leaves, Mt. Vernon, Skagit Co., Washington, September 20, L. W. Boyle. $\times 1$. E. Detail of C, a, showing enlarged lenticular perforations. $\times 3.5$. F. Apple leaf from Lynden, Whatcom Co., Washington, September 19, M. J. Forsell and A. J. Hansen. $\times 1$. G. Prominent spot on fruit of Ortley apple variety, Bay Center, Pacific Co., Washington, September 28, L. W. Boyle. $\times 3.5$. H. Detail of F, showing abundant dark fructifications of the conidial stage (c) H, a and H, b (inset) same as F, a, and F, b, respectively. $\times 12$. I. Spots on apple leaf, South Bend, Pacific Co., Washington, September 28, L. W. Boyle. $\times 1$. J and K. Spots from I showing small, although distinct pustules of the conidial stage; K, a, same spot as I, a. $\times 12$. Photographs by M. J. Forsell (A) and R. L. Taylor (B-K).



region of the spots was "lumier green" and "light lumier green." What may be of particular significance in connection with the survey is the single collection on quince of what is here tentatively identified as *Elsinoë piri*. The spotted-leaves concerned were collected at Olga, San Juan Co., Washington, on June 13, by C. Bodie of the U. S. Bureau of Entomology and Plant Quarantine.

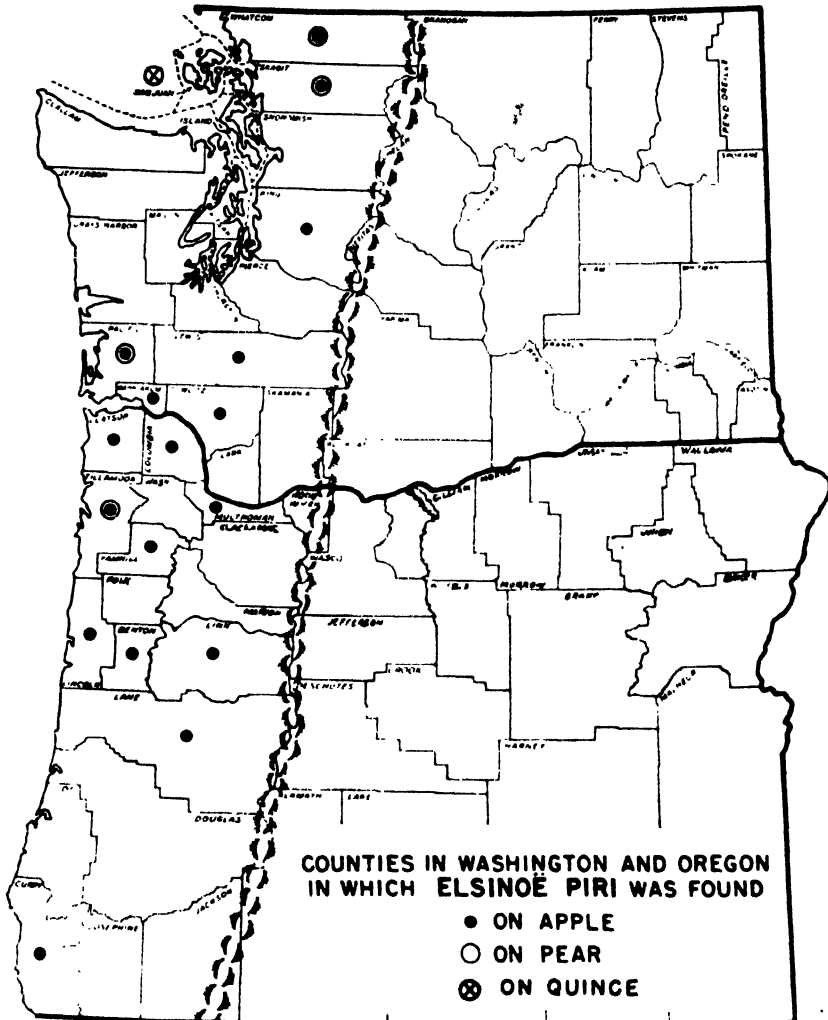


FIG. 2. Distribution of *Elsinoë piri* in Washington and Oregon in 1944.

All the specimens examined showed only the conidial stage, *Sphaceloma pirinum* (Peglion) Jenkins n. com. An isolation was readily made from the fruit illustrated in figure 1, C. On this fruit, vegetative growth of the fungus protruded through the enlarged lenticels (Fig. 1, E), as described by Osterwalder (9) in case of a fruit spot on the Jonathan apple variety.

This spot was subsequently identified by Jenkins and Horsfall (8) as the *Elsinoë* fruit spot. In general on the specimens examined, the conidial stage forms more or less extensive linear, sometimes branched, dark pustules protruding through fissures in the epidermis (Fig. 1, G, also illustrated at a magnification of 12 diameters in 4, Fig. 1, C). Conidia are rarely in evidence. Spots on pear leaves (Fig. 1, D) are similar to those on apple leaves; likewise they agree with Arnaud's (1, v. 2, p. 1059) description of a pear leaf spot in France, which has now been interpreted (4) as that caused by *E. piri*. The specimens and culture here utilized have been deposited in the Mycological Collections of the Bureau of Plant Industry, Beltsville, Maryland. Representative material also is included in fascicle 2 of "*Myriangiales selecti exsiccati*" by A. E. Jenkins and A. A. Bitancourt, now in process of issue.

The accompanying map (Fig. 2), showing counties in which the fungus was found in 1944, indicates its wide distribution west of the Cascade Mountains. Thus far there have been no identifiable findings of it elsewhere in North America.

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OCCURRENCE OF CURLY-TOP VIRUS IN MERISTEMATIC TISSUE

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(Accepted for publication February 21, 1946)

INTRODUCTION

The work of Bennett¹ and of Bennett and Esau² strongly indicates that the phloem is the tissue in sugar beet and tobacco in which the curly-top virus multiplies. It was demonstrated that the virus reaches its highest concentration in the phloem and that the virus is dependent on that tissue for translocation. Evidence was found that the virus sometimes occurs in the parenchyma but in relatively low concentrations. Anatomical observations indicate that exudate escaping from necrotic phloem may carry the virus into the intercellular spaces of the parenchyma. No explanation is known, however, for the fact that in the crown parenchyma where the cells are closely packed together there was found as much as or more virus than in the parenchyma with its larger intercellular spaces.

This paper reports evidence of virus multiplication in meristematic tissue.

MATERIALS AND METHODS USED IN TESTS ON ROOT TIPS OF SUGAR BEETS AND BEANS

Methods were designed to cut sections of sugar-beet and bean root tips below the protophloem sieve tubes which would contain the meristematic tissue of the growing point. To do this it was necessary to secure data on the distance from the tip of the root cap to the first protophloem sieve tube as well as to the growing point. This information was obtained by sectioning and measuring serial sections of root tips embedded in sets of five.

In order that some correlation could be made between size of root tips and distance to first protophloem sieve tube, the diameter of the tip was measured at the lower end of the first sieve tube. Approximately 500 root tips were examined, including those from both healthy and curly-top infected beets. The diameter of the healthy root tips at the level of the lower end of the first sieve tube varied from 0.31 to 0.66 mm. and the distance from the tip of the root cap to this point varied from 0.55 to 1.20 mm. The diameter of root tips from curly-top infected beets ranged from 0.21 to 0.51 mm. and the distance to distal end of the first sieve tube 0.52 to 1.13 mm.

With these data in mind it was possible to cut sections of root tips well outside the distal end of the first protophloem sieve tube which would contain mainly undifferentiated meristem of the growing point (Fig. 1). The fresh, living root tips were stained lightly with neutral red to make them visible when embedded. They were embedded in warm, soft paraffin in the

¹ Bennett, C. W. Plant-tissue relations of the sugar-beet curly-top virus. *Jour. Agr. Res. [U.S.]* 48: 665-701. 1934.

² ———, and K. Esau. Further studies on the relationship of the curly-top virus to plant tissues. *Jour. Agr. Res. [U.S.]* 53: 595-620. 1936.

following manner. Commercial parawax was used on account of its pliability at relatively low temperatures. The root tips were placed in a shallow groove in one side of a thin block of parawax. The soft wax was then gently folded to hold them firmly; or, sometimes, two thin blocks of softened paraffin were grooved together over a root tip. When the embedded tips were cooled in the refrigerator for a short time the hardened paraffin held the rootlet firmly without crushing it. By use of a hand microtome and sectioning razor, root-tip sections could be cut from 0.1 mm. up in length. The length of the root tip cut varied according to its diameter, so that an ample safety margin was left between the lowest section cut and the lower end of the first protophloem sieve tube. Large root tips were used. When a section was cut the paraffin was separated from it by means of dissecting needles. From 20 to 132 sections of root tips were used in different tests. These small sections were macerated in a 5 per cent sucrose solution and nonviruliferous leafhoppers were allowed to feed on this solution by using feeding cages of the type described by Bennett.³ The leafhoppers were then caged singly or in twos on susceptible test beets in the cotyledon stage.

RESULTS WITH SUGAR-BEET ROOT TIPS

By the use of the method described above for cutting these sections of the meristematic region of the diseased beet root tips a series of tests was con-

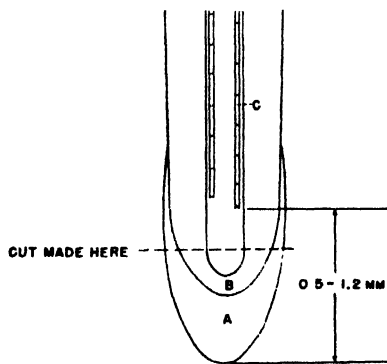


FIG. 1. Diagrammatic sketch of a longitudinal section of a sugar-beet root tip, showing the portion of the tip used in the tests made for the presence of virus. A. Root cap; B. growing point; C. protophloem sieve tube.

ducted. As indicated in figure 1, these sections, ranging from 0.2 to 0.4 mm. in length, were from the root-cap portions of the meristematic region and well below the first protophloem sieve tubes. In all 22 of these tests, using from 7 to 24 test beets in each test, positive results were obtained, with an average percentage of infection of 23.3.

Direct Feeding of Vectors on Beet Root Tips

For these tests, large, living root tips from sugar beets infected with curly-top virus were used. These were embedded singly in soft commercial

³ Bennett, C. W. Studies on properties of the curly-top virus. *Jour. Agr. Res.* [U.S.] 50: 211-241. 1935.

parawax leaving one-half millimeter or less of the root tip exposed. A short section of small glass tubing was placed over each of these protruding root tips and one or two leafhoppers put in the tube and the upper end closed with cotton. The vectors remained in the tubes 3 to 5 hours at relatively high temperatures. Some of the vectors died and it was difficult to determine the length of time the ones remaining alive had fed on the tips. Some of the leafhoppers remained alive although they did not feed during the 4 hours of the test. Infection was obtained in 3 out of 7 tests made. This again indicates the presence of virus in the meristematic tissue of the root tips.

The possibility that some leafhoppers may have reached a sieve tube when feeding was suggested. To answer this question, studies were made of leafhopper punctures in beet petioles as described by Bennett in 1934. Measurements were made of the depth of these punctures. Many of these punctures were on the side of the petiole away from the vascular bundles and so probably were of the maximum depth possible for the leafhoppers to penetrate. The punctures varied from 0.17 to 0.35 mm. where no phloem tissue was near. The measured distance from the extreme end of protophloem sieve tube to the surface of the root cap varied from 0.52 to 1.13 mm. In the large root tips used in these tests the distance averaged about 1 mm. It seems obvious, then, that the sieve tubes could not have been reached in the feeding process.

TESTS ON ROOT TIPS OF BEANS

Root tips of beans were tested because of their large size and the ease with which they can be grown. They averaged as large as the largest of the beet root tips. These tips were tested in the same manner as the beet tips. The sections of the root tip including the root cap were cut in lengths of 0.4 to 0.5 mm. These were tested for virus content. From 12 to 24 test beets were used in each of 15 tests and infection was obtained in all, with an average percentage of 32.6. As with the beet root tips, these results show that virus occurs in the meristematic tissue of bean root tips.

TESTS OF CAMBIUM FROM TOBACCO STEMS

Types of Plants Used

Two species of tobacco, *Nicotiana tabacum* L. var. Turkish, and *N. glutinosa* L., were used in these tests. When sampled for virus, these plants varied in age, condition of growth, and degree of severity of curly-top symptoms. When inoculated, there was not much difference in the size and age of these plants, which varied from 12 to 18 inches high. However, when cambium was taken from them for sampling, the Turkish tobacco plants were of four classes: (1) Rapidly-growing plants 12 to 15 inches high, 2 to 3 weeks after inoculation, and with severe symptoms; (2) plants 15 to 24 inches high, 3 to 5 weeks after inoculation, and with symptoms past maximum severity; (3) older plants about 24 to 30 inches high, 6 or more weeks after inoculation, showing some recovery; (4) old plants about 36 inches high, 8 or more weeks

after inoculation, and in an advanced stage of recovery with almost no symptoms on the young upper leaves. The *N. glutinosa* plants used in the experiments varied correspondingly in age, size, and length of time after inoculation.

Methods

The bark of a rapidly growing tobacco plant may be readily removed, leaving a layer of cambium tissue on the woody stem. This meristematic

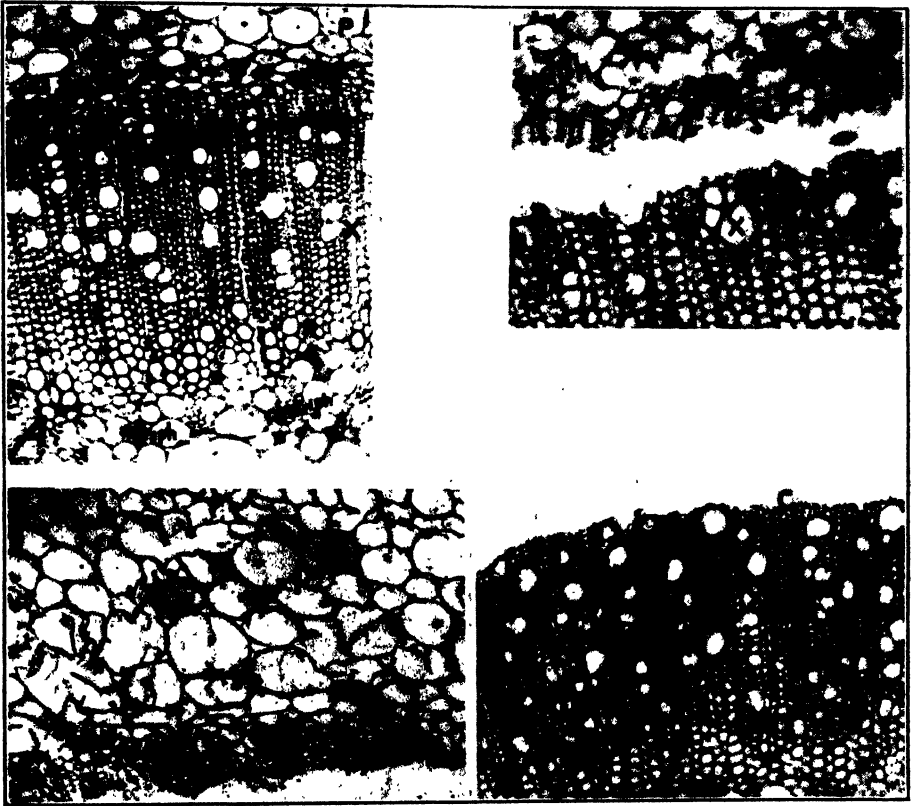


FIG. 2. Transverse sections of tobacco stems: A, Parenchyma cells (p) of bark, external primary phloem (eph), cambium ring (c), point where bark separates from (sp) woody cylinder of xylem (x) and internal primary phloem (iph); B, bark separated from woody cylinder of xylem (x) and showing external primary phloem (eph) in bark at upper edge of cambium layer (c); C, bark of tobacco stem showing external primary phloem (eph) in it; D, woody cylinder showing xylem area (x) and outer edge free from internal primary phloem tissue after bark is peeled off, (c) thin cambium layer.

tissue was scraped from 3- to 4-inch sections of the tobacco stems and the scrapings were suspended in a 5 per cent sucrose solution. Nonviruliferous leafhoppers were allowed to feed on sucrose suspension of the scrapings by using the same type of feeding cage employed with the tests on macerated root tips.

By this method there was little chance of contamination from vascular tissue, as is evident by examination of figure 2. Figure 2, A, is a transverse

section of a tobacco stem showing the bark with external primary phloem, cambium layer, woody xylem, and internal primary phloem. Figure 2, B, illustrates how the bark comes loose from the woody cylinder, leaving only a thin layer of cambium on it and taking all the external primary phloem with the bark. Figure 2, C, shows a section of bark with the external primary phloem and most of the cambium layer pulled off with it. Figure 2, D, is the woody cylinder left after removing the bark with only a thin uneven layer of cambium tissue left on it. Both freehand and prepared sections were examined under the microscope after removing the bark to check possi-

TABLE 1.—*Tests for occurrence of curly-top virus in cambium of Nicotiana tabacum*

Experiment number	Test beets inoculated	Test beets infected	Disease condition of tobacco plants when cambium was tested
	Number	Per cent	
1	24	70.8	Young plants 12 to 15 inches high, inoculated for 2 to 3 weeks, and with curly-top symptoms of maximum severity.
2	18	44.4	
3	20	65.0	
4	28	64.2	
5	20	50.0	
6	20	35.0	Plants 18 to 24 inches high, inoculated for 3 to 5 weeks, and with curly-top symptoms a little past maximum degree of severity.
7	18	27.3	
8	20	30.0	
9	20	50.0	
10	24	25.0	Plants 24 to 30 inches high, inoculated for about 6 weeks, less severe symptoms than in the experiments 6 to 9, and first definite signs of recovery evident.
11	20	8.5	
12	20	15.0	
13	20	30.0	
14	20	10.0	Old plants about 36 inches high, inoculated for 8 weeks or more, in an advanced stage of recovery.
15	20	0.0	
16	20	0.0	
17	20	10.0	
18	20	0.0	
19	20	5.0	
20	22	13.6	

bility of some outer phloem tissue remaining on the peeled stem. In every case examined the outer phloem came off with the bark.

Results

The results of these tests with cambium from *Nicotiana tabacum* and *N. glutinosa* are in tables 1 and 2. The concentrations of virus in the cambium were highest in the case of the youngest plants with curly-top symptoms of maximum severity. Apparently the virus concentration in the cambium decreased as the plants recovered.

DISCUSSION

The curly-top virus has certain advantages for the determination of its presence in meristematic tissues. This is due to its inability to infect plants by juice contact, as is the case with some of the mosaic viruses. Since its rapid translocation is limited to the phloem and since there appears to be

little if any of it in parenchyma tissue, there is little chance of contamination in dissecting out the meristematic tissue for testing.

The question arises, why does the virus exist in the meristematic tissue of diseased plants, while there is little or none of it in the parenchyma? In parenchyma derived from invaded meristem the virus must be inactivated or its multiplication stopped.

Relatively high concentrations of virus were found in the cambium near the lower ends of stems of tobacco plants inoculated in the tops. Bennett⁴ in his general review of movement of viruses states that present evidence indicates viruses will not move faster than 1 to 2 mm. a day through paren-

TABLE 2.—*Tests for occurrence of virus in cambium of Nicotiana glutinosa*

Experiment number	Test beets inoculated	Test beets infected	Disease condition of tobacco plants when cambium was tested
	<i>Number</i>	<i>Per cent</i>	
1	20	50.0	Young plants 12 to 15 inches high, inoculated for about 2 weeks, symptoms at maximum degree of severity.
2	16	50.0	
3	20	60.0	
4	20	70.0	
5	20	65.0	
6	20	60.0	
7	18	33.3	
8	16	62.5	
9	12	66.6	
10	13	30.8	Plants 15 to 20 inches high, inoculated for 3 to 5 weeks, symptoms past maximum degree of severity.
11	20	30.0	
12	20	35.0	
13	20	20.0	
14	20	10.0	Plants 24 to 30 inches high, inoculated for 6 to 8 weeks, in an advanced stage of recovery from curly top symptoms.
15	20	20.0	
16	20	10.0	
17	20	0.0	
18	20	0.0	
19	20	10.0	
20	20	0.0	

chyma cells. Such a rate of movement would not be fast enough to account for the virus being in the cambium of the lower end of the tobacco stem in such a relatively short time after inoculation even though these plants were only 12 to 18 inches high when inoculated. The probable explanation is that the virus passes from the phloem into the cambium and multiplies in this meristematic tissue. The course of developments in root tips is probably similar.

It seems strange that with fairly high concentration of virus in the cambium and undifferentiated region of the root tips no injury to these meristematic tissues has been observed. Necrosis in the phloem of infected plants is the rule. The few cases of cell degeneration noted by the writer⁵ in the

⁴ Bennett, C. W. The relation of viruses to plant tissues. Bot. Rev. 6: 427-473. 1940.

⁵ Lackey, C. F. Curly-top virus in root tips of sugar beets and beans. (Abstr.) Phytopath. 28: 671. 1938.

apparently undifferentiated region of root tips were cells directly below the extreme ends of sieve tubes. The location of these affected cells suggests that they had passed beyond the meristematic stage. Injury to differentiated cells adjacent to the phloem in diseased plants has been commonly observed.

DIVISION OF SUGAR PLANT INVESTIGATIONS,

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INJURY TO APRICOT LEAVES FROM FLUORINE DEPOSIT

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Injury to dairy pastures and orchards in the vicinity of an aluminum reduction plant was reported in 1943 and 1944. The injury to dairies consisted of the deposit of fluorine on alfalfa and other pasture crops where dairy herds were being kept. This deposit was reported by State authorities as being of sufficient amount to be dangerous to cattle feeding in the exposed fields adjoining the reduction plant and in the direction of the prevailing winds.

Vegetation in the area adjoining the reduction plant suffered to a varying degree. Many of the broad-leaved plants were blackened, and a few English walnut trees and apricot trees within one-half mile of the plant were almost defoliated by the end of July, 1944. Apricot foliage still remaining on the trees was a dull red brown, and the outer edges of the leaves were dead.

Examinations were made at intervals and foliage and fruit sampled from a large orchard property 1½ miles from the aluminum reduction plant, and directly in line with the prevailing wind. The orchards consisted of 60 acres of White Adriatic figs, 40 acres of Tilton apricots, and 5 acres of Elberta peaches. The part of the fig orchard adjoining the reduction plant was severely defoliated even in August. This varied because the soil is spotted, but there was less defoliation and a better growth of both fruiting wood and the current crop in the half of the orchard furthest removed from the fumes of the plant. The 1943 crop was lighter than in the two previous years, while the growth of fruit wood was shortened and frequently the terminal buds were injured, which resulted in a "witch broom" effect. No discoloration of foliage was noted.

The apricot orchard was very conspicuous from the type of injury occurring. The area adjoining the plant was about 60 per cent defoliated the first part of August. Most of the leaves remaining on the tree were injured and the brown, dead parts of the leaf had an appearance of fire damage. Much gumming was found on the young twigs, and gum pockets were at the crown of some 5-year-old trees. Defoliated areas of 5 to 16 inches were noted on a large proportion of the twigs that made up the bearing wood. Beyond this defoliated area the younger leaves had varying amounts of brown, dead tissue. This type of injury was noted throughout the orchard but was more pronounced adjoining the reduction plant and in spots through the older orchard of 11 to 13 years. Unfavorable soil conditions in gravel spots in the sub-soil or in spots where the soil was too heavy, resulted in an intensification of the symptoms. The 1945 crop was only about 50 per cent of normal; its crop failure apparently being due to the early defoliation and bud injury occurring in 1944. The amount of fluorine present on the leaves was found by washing them in a weak acid solution and determining the fluorine in the

washings by chemical analysis. The range of fluorine found on leaves, taken at random through the fig and apricot orchards, was from 247 to 403 parts per million.

Apricot leaf injury first appeared 2 to 3 weeks after the trees were in leaf. The first symptom was a wilting of the edges, which became progressively more pronounced for about 10 days by which time the tissue was brown and dead. The width of the injury varied from $\frac{1}{4}$ to $\frac{3}{8}$ of an inch, but except in the most severe cases, a varying amount of normal tissue was noted along the central vein. Figure 1 shows the form of injury commonly noted. This type of injury was seen throughout the season. The growing tip of the shoot developed normally and only leaves almost full grown were injured. The damage continued for two years, unchecked, until the plant was shut

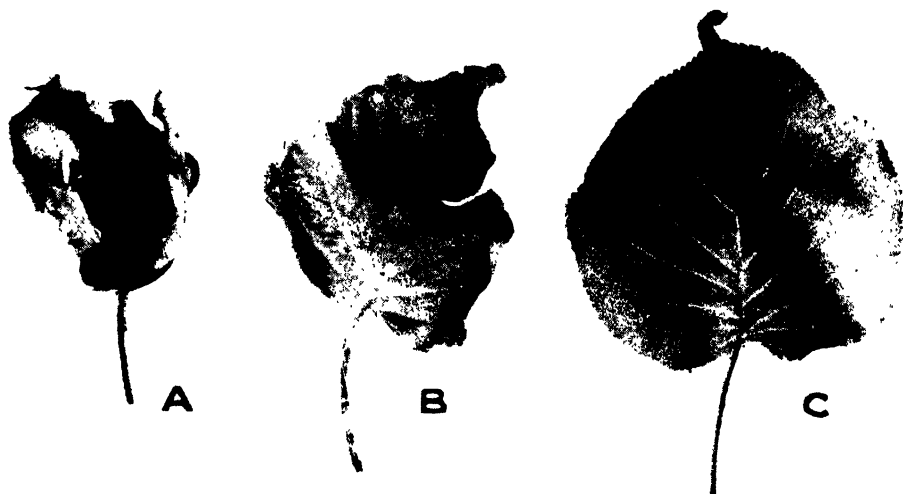


FIG. 1. Apricot leaves. A. Leaf, injured by fluorine deposit, with dead marginal areas attached. B. Injured leaf from which dead areas have fallen off. C. Normal leaf, not injured.

down in August, 1944. Within three weeks following the closing of the plant new growth was normal and no injury to leaves was found afterwards. Similar injury to apricots was noted in a decreasing degree for 8 miles to the windward of the plant. The same type of injury was found on lilac, certain types of blackberry, roses, hydrangea, and a plum tree. No injury was noted on the young peach orchard, although it adjoined the aluminum reduction plant. The injury resulting from the fumes of the reduction plant is distinctly different from boron injury as commonly reported. The first symptom of boron injury is a weakened or dead terminal growth sometimes resulting in dead spikes. Contrasting with this type of injury, the fluorine damage to apricot was on mature or practically full grown leaves. The deposit, probably solid particles with fluorine adsorbed, accumulated on the leaves for two weeks or longer before pathological symptoms appeared. Injury was no doubt intensified by the lack of rain during the dry summer, as in a humid climate the deposit would have been washed off by rain.

Sufficient moisture was present, however, in the dew and fog to dissolve any form of soluble fluorine present.

The presence of fluorine in the fumes emanating from an aluminum reduction plant may result from a disturbance of equilibrium in the electrolytic bath where the bauxite or feldspar ore is treated. A process commonly used in recovering alumina is to fill the pots with a mixture of cryolite ($3\text{NaF} \cdot \text{AlF}_3$) and the aluminum ore. The cryolite is kept in a molten condition and the ore added at intervals. A disturbance of the balance between the fused cryolite and the aluminum ore results in freeing a certain amount of fluorine which would be released through the stacks. The control of flue gases, including the released fluorine, is a practical method of avoiding such injury in fields adjoining reduction plants.

SAN FRANCISCO, CALIFORNIA.

SEASONAL CHANGES IN BIOLOGICAL EQUILIBRIA INVOLVING TWO CHONDRIOSOMAL SYSTEMS IN VARIEGATED *HOSTA*

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(Accepted for publication March 12, 1946)

Plastids are known to have certain properties of living organisms (5, 7, 9). They arise only from pre-existing plastids or plastid-forming mitochondria, multiply by fission, and have a definite heredity (5, 7, 9). It has been observed that mutated plastids are the cause of certain types of leaf variegation (7, 9, 12). Recently it has been further shown that these abnormal plastids have an effect on the cells that contain them which is in certain respects remarkably virus-like (11). Thus variegation-inducing plastids (hereafter the abbreviation *vi*- will be used) by their presence in the cell lead to characteristic changes in the development and function of the normal plastids, cell maturation, and cell metabolism (11). Furthermore observed gradations in structural complexity and pathogenic action of the *vi*-plastids in a large number of variegations have suggested more than superficial similarities between variegations of plastid origin, and those due to certain types of viruses (2, 11). Demonstration of ribosenucleoprotein in virus-free plastids (2), confirming observations by Menke (8), furnishes a probable chemical basis for the theoretical heredity-controlling plastogene of Imai (7).

In the origination of variegation it seems probable that the initial mutation would in most cases occur in a single plastid or mitochondrion. In any case the establishment of variegation requires that the mutated plastid-forming mitochondria be able to multiply in the presence of the normal or parent plastidome. Field observations of a plastid-induced variegation in *Hosta* revealed that a seasonal masking of symptoms occurred which was in many respects strikingly like seasonal masking of symptoms in certain virus diseases. Because of the previously demonstrated similarities in cellular pathology in plastid-induced variegations, and certain virus diseases (11), this behavior of variegated *Hosta* was of special interest. Furthermore the material seemed favorable for a study of factors which modify the quantitative relationships between the two plastid systems of a variegated plant during leaf development. The present paper reports results of such a study.

MATERIALS AND METHODS

A white variegated clone of *Hosta japonica* Asch. and Graeb. (Fig. 1) was grown out of doors or forced in the greenhouse during winter. Material for cytological examination was fixed by aspiration in freshly prepared neutral formalin solution (10 ml. of a fresh distillate of a mixture of 37 per cent formaldehyde and an excess of NaHCO_3 added to 90 ml. of water) and

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examined without sectioning. Some preparations were kept for later observation by replacing the formalin solution with 10 per cent glycerine. While sectioned material was used, much better results could be obtained with the intact leaf. Yasui (12) employed a similar technic with variegated *Hosta*.

MACROSCOPIC SYMPTOMS OF VARIEGATION

Symptoms of variegation ranged from a uniform sectorial pattern to a highly irregular mosaic of variegated and normal-appearing tissues (Fig.

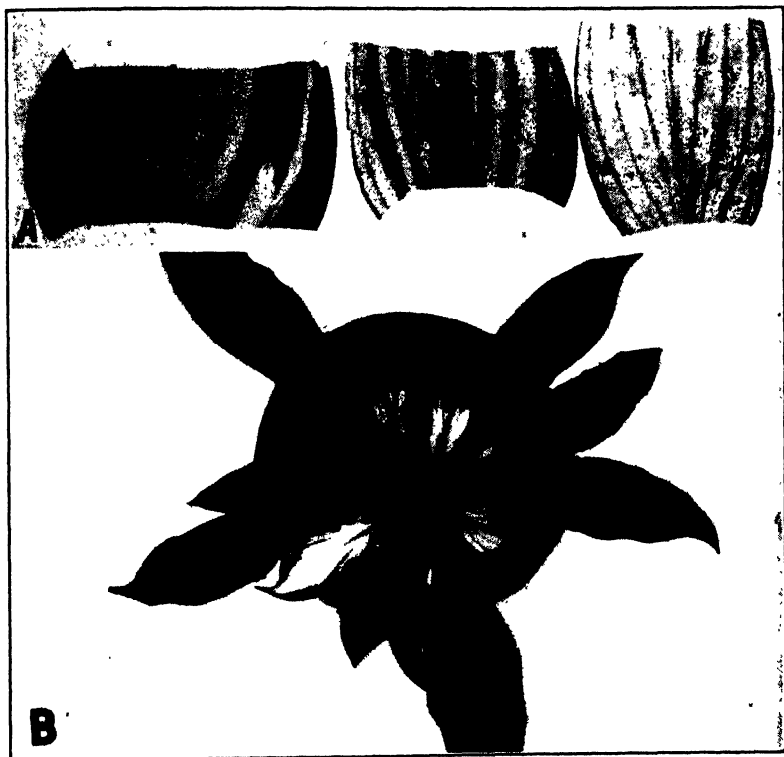


FIG. 1. Variegated *Hosta japonica* Asch. and Graeb. A. Three symptom patterns in leaves formed in spring or early summer. Left to right: sectorial, sectorial-mosaic with green veins, highly irregular mosaic. B. Influence of temperature on symptom pattern. Oldest leaves (green); developed in warm greenhouse, intermediate leaves (variegated strongly); developed in cool greenhouse, youngest leaves (green); developed outside during summer.

1, A). These patterns are due to the distribution of *vi*-plastids which are without visible pigmentation and distinctly smaller than the green plastids (Fig. 2). The contrast between the two types of plastids is always marked, and heterochondric cells (cells containing both types of plastids) are readily distinguished (Figs. 2 and 3). A somewhat similar variegation in *Hosta* has been described by Yasui (12). She noted that the median longitudinal parts of leaves formed in the spring were yellow whereas summer leaves were greenish yellow. Her cytological descriptions, in so far as they cover the

same ground, are in essential agreement with ours. In our clone of variegated *Hosta*, leaves formed early in the growing season were strongly variegated whereas leaves formed during the summer were successively less variegated until some of them appeared macroscopically free of variegation. However, leaves formed in September were often almost as strongly variegated as those formed during the spring. Plants forced from January to March, in a greenhouse held at temperatures approximating those of mid-summer, developed leaves with strongly masked variegation (Fig. 1, B). Leaves developed in a cooler greenhouse on the other hand were strongly

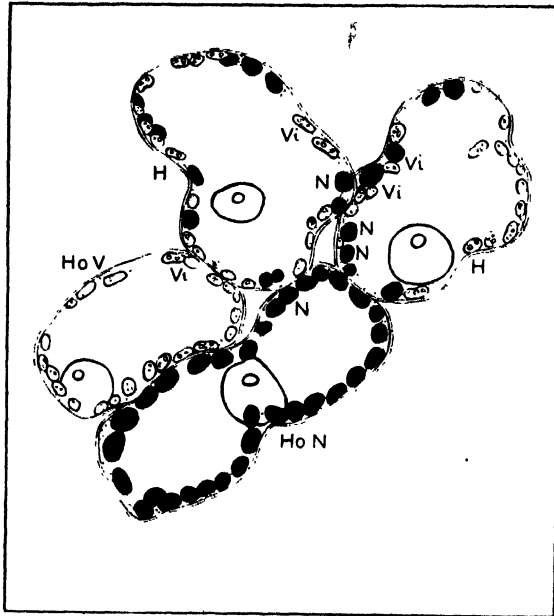


FIG. 2. Four cells from a young summer leaf (July 7) of variegated *Hosta japonica* Asch. and Graeb. These cells occurred in one of the very restricted areas with visible symptoms of variegation. H, heterochondric cell (variegation inducing plastid labeled vi); HoV, homochondric variegated cell; HoN, homochondric normal cell. (Approx. 700 \times .)

variegated. It should be emphasized that throughout the season no change in the symptom pattern of leaves already formed was observed. The masking of variegation apparently took place during leaf ontogeny.

The mesophyll immediately adjacent to the vascular tissues sometimes remained green although the interveinal tissues were nearly or completely white (Fig. 1, A). Microscopical examinations throughout the growing season demonstrated the cytological basis for these variations in symptoms of variegation.

CYTOLOGY OF VARIEGATED TISSUES

Totally variegated areas occurred extensively in spring and early summer leaves. Most cells in such areas contained only variegation-inducing (vi-) plastids. Spring leaves with clear-cut sectorial variegation (Fig. 1, A)

were characterized by a preponderance of cells containing only one type of plastid with sharp boundaries between green and white tissues (Fig. 3, C) whereas spring leaves with the irregular mosaic variegation (Fig. 1, A) contained many heterochondric cells (Fig. 3, A and E). In the latter, *vi*-plastids tended to predominate. As the season progressed, successively formed leaves contained variegated areas which were more and more suffused with green. Microscopically this was paralleled by a gradual increase of normal

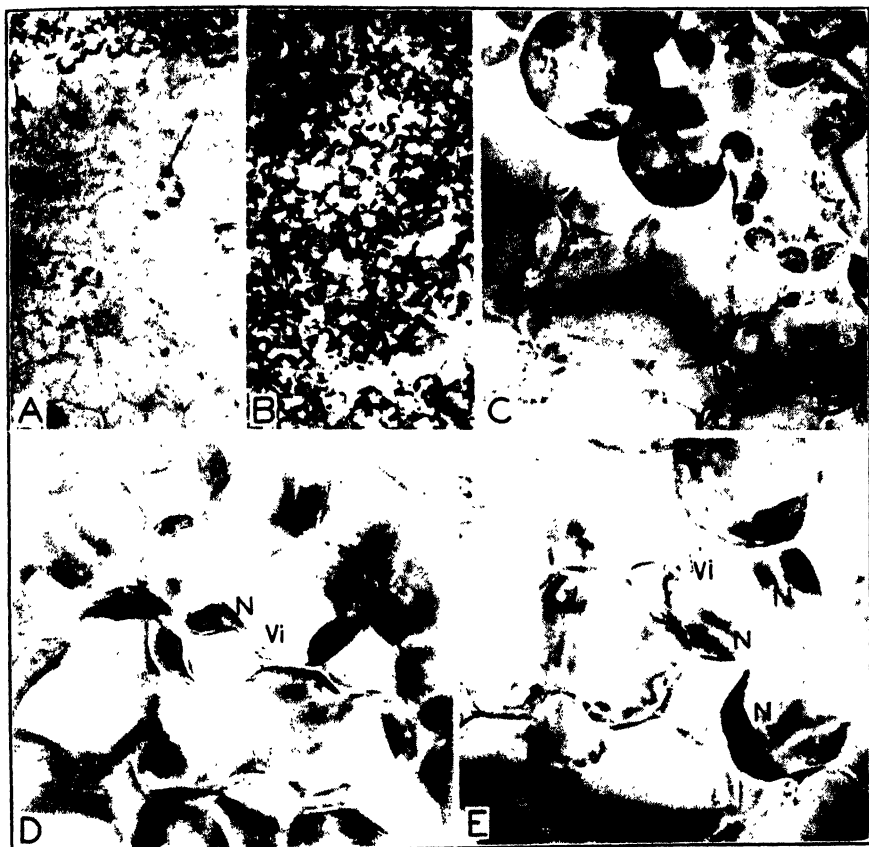


FIG. 3. Photomicrographs of unstained and unsectioned leaves of variegated *Hosta*. A. Heterochondric and homochochondric variegated cells adjacent to homochochondric normal area in spring leaf. B. Heterochondric area in summer leaf. C. Abrupt transition between homochochondric variegated and normal cells of spring leaf. D. Heterochondric cells of summer leaf. E. Heterochondric cells of spring leaf (*vi*-plastids in D and E labeled—*vi*, normal type—N). (Magnifications: A and B, approx. 140; C, D, and E, approx. 900.)

plastids in the abundantly occurring heterochondric cells (Fig. 3, B and D). This decrease in the number of the *vi*-plastids resulted in the formation of large numbers of cells which were free of *vi*-plastids or contained only a few (Fig. 4). This constituted a reversal of the behavior noted earlier in the season. Yasui (12) attributed the deepening of green color in summer

leaves to a growth in size rather than to a change in number or growth rate of normal type plastids in cells with both types of plastids (heterochondric cells). This, however, does not explain the phenomena observed in our material. We did find the plastids of old normal leaves to be much larger

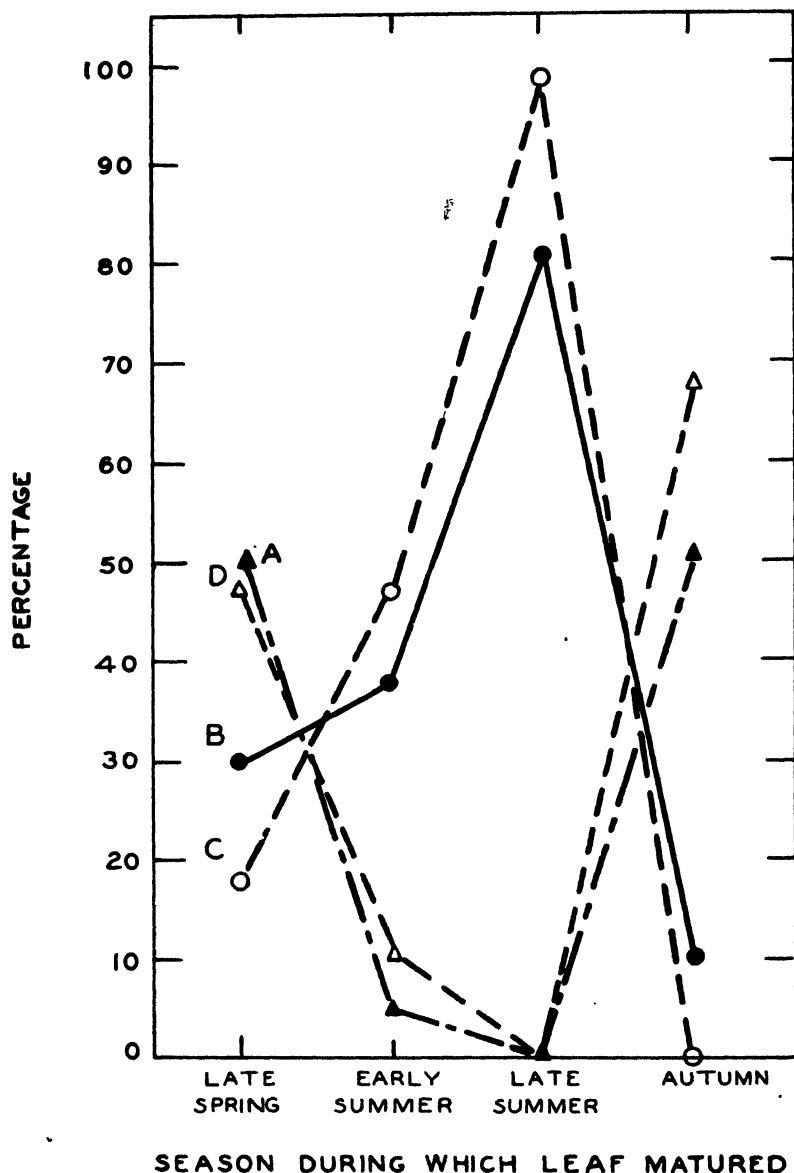


FIG. 4. Influence of season on development of variegation in *Hosta japonica* Asch. and Graeb. A. Approximate percentage of total leaf area variegated. B. Average percentage of a normal green plastid complement in heterochondric cells in measured area (20-25 cells counted). C. Percentage of cells in measured area (of 100 or more cells) that were apparently homocondric normal. D. Percentage of cells in measured area that were homocondric variegated. One typical leaf representative of each season was selected for each set of measurements.

than those of young leaves. Similarly the green plastids of heterochondric cells become abnormally large (2 to 4 times) on aging of the tissues (Fig. 3). These changes in size, however, did not account for the seasonal or high temperature masking, which is due to an increase both in the number of normal type green plastids in the heterochondric cells (Fig. 3, B) and in the number of mature, apparently normal cells (Fig. 4). Some leaves formed during summer appeared macroscopically free of variegation. However, on microscopical examination occasional cells with only one or two *vi*-plastids could always be found in addition to cells in which only normal plastids could be found. In no case was there evidence of plastids of intermediate type. With the reappearance of variegation in September and October many heterochondric cells with large numbers of *vi*-plastids were again observed. Some leaves had large areas of totally variegated cells only.

Cytological examination has shown that there is a tendency for the tissues along the veins occasionally to remain green. Apparently in heterochondric cells close to the vascular tissues conditions are more favorable for the development of normal plastids than for the *vi*-type. Often a rough gradation in the number of *vi*-plastids in the mesophyll cells adjacent to the veins was observed. Thus cells located close to vascular elements appeared either normal or had very few *vi*-plastids, whereas cells further removed from the veins had progressively more *vi*-plastids until homochondric variegated cells occurred mid-way between adjacent veins. The small secondary veins sometimes exerted an influence of a similar nature on the mesophyll (Fig. 1, A). Spring leaves with clear-cut sectorial variegation (Fig. 1, A) were characterized by a preponderance of homochondric cells, both normal and variegated (Fig. 3, A). In leaves of this type there was often little or no observable influence of the veins on cells of the white areas of the leaves. The distribution pattern of the *vi*-plastids, especially the manner in which the veins limit variegated areas, is suggestive of limited intercellular migration of *vi*-plastid forming mitochondria during certain stages of leaf ontogeny as previously noted in other variegations (11). However, proof of such migration must await further work.

DISCUSSION

These studies emphasize the importance of both the external environment (*e.g.*, temperature) and the internal environment (*e.g.*, vascular influences) on the quantitative relationships between the variegation-inducing and normal plastid systems of the cell. The results allow the conclusion that these relationships are in the nature of a biological equilibrium between the cell and the two plastid systems, although the precise nature of this balance remains to be determined. These relationships may be compared with the interactions that exist between a virus and a normal chondriosomal system of an infected cell (10). The interference with the development of the plastids characteristic of certain viruses is well known (11). Since plastids can be considered specialized mitochondria (5) the results might be suggestive

of an approach to a group of animal neoplastic diseases where the existence of similar systems in the cell has been postulated (1, 3, 4, 6).

SUMMARY

White variegated areas of *Hosta japonica* Asch. and Graeb. were due to the presence of colorless plastids which, under certain conditions, multiplied at rates different from the normal plastids in cells which contained both types (heterochondric cells).

In leaves formed in spring or autumn the variegation-inducing plastids apparently suppressed the multiplication of the normal plastids in heterochondric cells and the leaves were strongly variegated. The reverse was true during summer when "masking" of symptoms occurred in successively formed leaves.

The vascular tissues of the leaf exerted an influence on adjacent mesophyll of such a nature that multiplication of normal plastids frequently seemed to occur at the expense of the *vi*-plastids in heterochondric cells regardless of season.

The bearing of these results on cancer and virus problems is discussed.

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PHYTOPATHOLOGICAL NOTES

Flag Smut of Wheat in Mexico.—Flag smut of wheat, caused by *Urocystis tritici* Koern., has never been reported from Mexico prior to 1945. It was first encountered in the region of Zitacuaro, State of Michoacán, on April 27, 1945, and appeared to be distributed throughout a number of fields lying within an area of approximately 10 square miles.

Circumstantial evidence indicates that the causal organism was brought into Mexico on wheat imported from Australia. This wheat was destined for milling, but evidently some found its way into the hands of growers and was used for seed. It is possible that other diseased fields existed during the current year in other parts of the Republic, but no positive evidence was obtained outside of Michoacán.

The Dirección General de Agricultura, Secretaría de Agricultura y Fomento, carried on an eradication campaign in the Zitacuaro area, and a quarantine has been established to prevent the movement of seed wheat from this area to other regions. A campaign of seed treatment has also been begun as an aid in preventing the establishment of flag smut.

The vast majority of the wheats grown in Mexico are susceptible to flag smut. In the event that the eradication campaign is unsuccessful and the disease becomes well established within the country, it will be necessary to begin a program of introduction and breeding for the ultimate selection of resistant varieties.—N. E. BORLAUG, J. G. HARRAR, and E. C. STAKMAN, The Rockefeller Foundation, Mexico D. F.

The Effect of Depth of Planting on the Emergence and Survival of Blue Lupine.—One of the most serious troubles in the growing of blue Lupine (*Lupinus angustifolius* L.) as a winter cover crop in Florida is the loss of plants in the seedling stage. After the plants attain a height of 6 to 8 inches, few fail to grow to maturity. Among the organisms isolated from the dead or dying plants, *Rhizoctonia* spp. far outnumber all others. The host-parasite relationship and the factors affecting this relationship are not fully understood. However, it has been observed that fewer plants are lost when conditions favor quick emergence and rapid growth of the Lupine seedlings. One factor affecting the rapidity of emergence and subsequent growth is the depth of planting.

In an attempt to evaluate the effects of the depth of planting on losses from disease, field soil was taken from the surface three inches and put into flats. Sixteen flats were prepared and moved into the greenhouse, where 200 blue Lupine seeds were planted in each flat. In 8 of the flats, the seeds were planted one inch deep while in the remaining 8 flats the seeds were planted two inches deep. Five days after planting 94.1 per cent of the seeds planted one inch deep had emerged; the peak of emergence of 54.7 per cent for the deeper planting was recorded 10 days after planting. A photograph

taken 14 days after planting demonstrates the difference between the two depths of planting in the stand and growth of the seedling plants (Fig. 1).

Some plants were killed by *Rhizoctonia* in all plantings: in the shallow plantings, the seedlings were killed in small localized areas, while only a few scattered plants escaped in the deeper plantings. Twenty-one days after

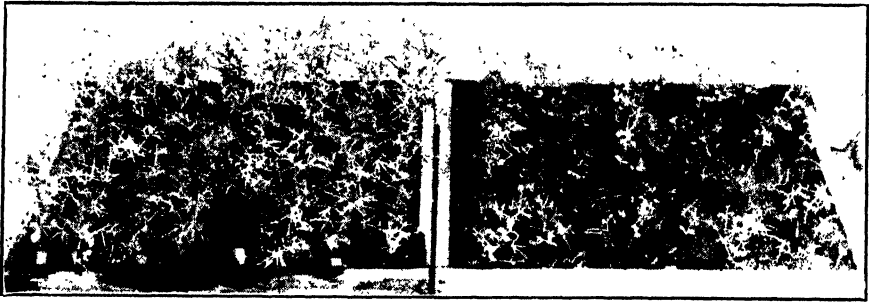


FIG. 1. Blue Lupine plants showing the effect of depth of planting upon stand and growth of seedling plants. Left, seeds planted 1 inch deep. Right, seeds planted 2 inches deep.

planting, when the plants in the shallow plantings had attained an average height of 8 inches, 70.7 per cent of these plants appeared healthy, while only 6.5 per cent of the plants in the deeper plantings were alive.—PHARES DECKER, Florida Agriculture Experiment Station.

A Type of Internal Necrosis of the Potato Tuber Caused by Psyllids.—Manson and Sanford are reported to have found in Canada an internal necrosis of the potato tuber caused by the tomato psyllid.¹ In a recent study of spindling sprout in the White Rose potato² an opportunity was afforded to examine for internal symptoms several dozen tubers from plants artificially infested with psyllids. The tubers had been stored at room temperature for about 6 months and either had produced spindling sprouts or failed to sprout at all. On cutting, all these tubers had a characteristic discoloration in the vicinity of the smaller vascular elements. A similar number of control tubers from noninfested plants were free from internal necrosis. The discoloration extended throughout the length of the tuber but was somewhat more prominent at the stem end. On closer examination the discoloration was found to be due to discontinuous dark flecks (Fig. 1, A) rather uniformly distributed in cross section from the main vascular ring to near the center of the tuber. In early stages under magnification an irregular black and white pattern was evident within the flecks. With still higher magnification the cells were seen to contain dark bodies irregular in size, and amorphous to smooth and spherical in appearance. (Fig. 1, B). In later stages, the flecks became more readily discernible macroscopically because

¹ Sanford, G. B., and J. G. Grimble. Observations on phloem necrosis of potato tubers. *Canad. Jour. Res.* 22: 162-170. 1944.

² Snyder, W. C., H. E. Thomas, and S. J. Fairchild. Spindling or hair sprout of potato. *Phytopath.* 36: (In press). 1946.

of the brownish cast due to cell necrosis, but they lost the characteristic, discrete, dark, intracellular bodies.

It is hoped that the distribution of the flecks and their distinctive microscopic appearance will prove to be of diagnostic value for psyllid yellows where this type of internal necrosis of the tuber occurs.

In other experiments in which spindling sprout was induced by infesting the mother plant with psyllids, internal necrosis was not observed. However, in these instances either the amount of toxin taken up by the tubers



FIG. 1. Internal necrosis of potato tubers from psyllid-infested plants. A, longitudinal and cross sections of affected tubers. B, detail of one of the flecks in A, before complete necrosis, to show several of the dark, intracellular bodies, 180 \times (photograph by Catherine Roberts).

may have been less or the tubers may not have been held in storage for a sufficiently long time.

Our evidence does indicate that the kind of internal necrosis illustrated in figure 1 may occur, like spindling sprout, as a symptom of psyllid yellows when tubers produced on heavily infested plants are held in storage.—WILLIAM C. SNYDER, H. EARL THOMAS, and S. J. FAIRCHILD, Division of Plant Pathology, University of California, Berkeley, California, and Mettler Research Laboratory, Santa Maria, California.

Spermogonia versus Pycnidia in Mycosphaerella brassicicola.—A pycnidial stage of *Mycosphaerella brassicicola* (Fr.) Lindau has long been accepted, with the ascigerous stage, as a cause of infection and spread of the

ringspot disease of crucifers. The so-called pycnidial stage occurs abundantly and universally in affected plantings, and has been referred to as a *Phyllosticta*.¹ While making a survey of cruciferous crops for diseases^{2,3} it was observed that ringspot lesions were always evenly spaced and distributed over the affected leaf at random (Fig. 1, A) in a manner expected only where the inoculum is wind-borne. Moreover, no instances were found where secondary lesions were grouped about primary lesions in the type of tear-stain infection usually obtained from water-borne inoculum, such as is the case with the *Phoma* and *Alternaria* leaf spots of crucifers. Yet it is the arrangement of great numbers of the so-called pycnidia, and later of perithecia, in each lesion in concentric circles, which is largely responsible for the characteristic symptom and name of the disease.

In the winter of 1945, an examination of numerous infected, living leaves of diseased cabbage plants from the field showed the presence of pycnidium-like bodies and pycnidium-like ooze which corresponded to that previously described for the pycnidial stage.⁴ Some of the ooze was collected and mixed with an ascospore suspension prepared from perithecia produced on dead, infected leaves found on the ground beneath diseased plants. This mixed suspension was placed on potato-dextrose, cornmeal, oatmeal, and water agars and incubated both at room temperature, and out-of-doors at Berkeley in winter. Drops of the mixed suspension were also placed on glass slides, with and without bits of healthy cabbage leaves, and on healthy leaves, in moist chambers, under both environments. In all cases germination of the ascospores was obtained within 24 hours (Fig. 1, B) but none of the so-called pycnosporos produced any growth in 7 days. Weimer also reported failure to germinate the pycnosporos. Furthermore his illustrations of symptoms show only evenly spaced lesions.

Twenty inoculations of detached cabbage leaves were made with ascospores and twenty with the ooze referred to above. Typical ringspot was obtained only where the ascospore inoculum was used, and only where the leaves were incubated out-of-doors.

Twenty single-spore cultures were made from ascospores, and 20 cultures from ooze, on potato-dextrose, cornmeal, and oatmeal agars, and placed out-of-doors. None of the cultures prepared from the ooze grew, whereas all of the single ascospore transfers developed into colonies, and all produced in time the so-called pycnidia, and ooze. Moreover, all the cultures on cornmeal agar produced, in addition, mature perithecia. It is believed this is the first time this fungus has been carried through its complete life-cycle in culture. Since these were single ascospore cultures, *Mycosphaerella brassicicola* is obviously a homothallic fungus.

¹ McAlpine, D. Fungus diseases of cabbage and cauliflower in Victoria, and their treatment. Victoria Dept. Agr. Unnumbered Bul. 38 p. 1901.

² Snyder, W. C., and K. F. Baker. Diseases of seed cabbage in California. U. S. Dept. Agr., Pl. Dis. Repr. 27: 394-398. 1943.

³ Snyder, W. C., and K. F. Baker. Diseases of seed cauliflower in California. U. S. Dept. Agr., Pl. Dis. Repr. 29: 248-253. 1945.

⁴ Weimer, J. L. Ringspot of crucifers caused by *Mycosphaerella brassicicola* (Fr.) Lindau. Jour. Agr. Res. [U.S.] 32: 97-132. 1926.

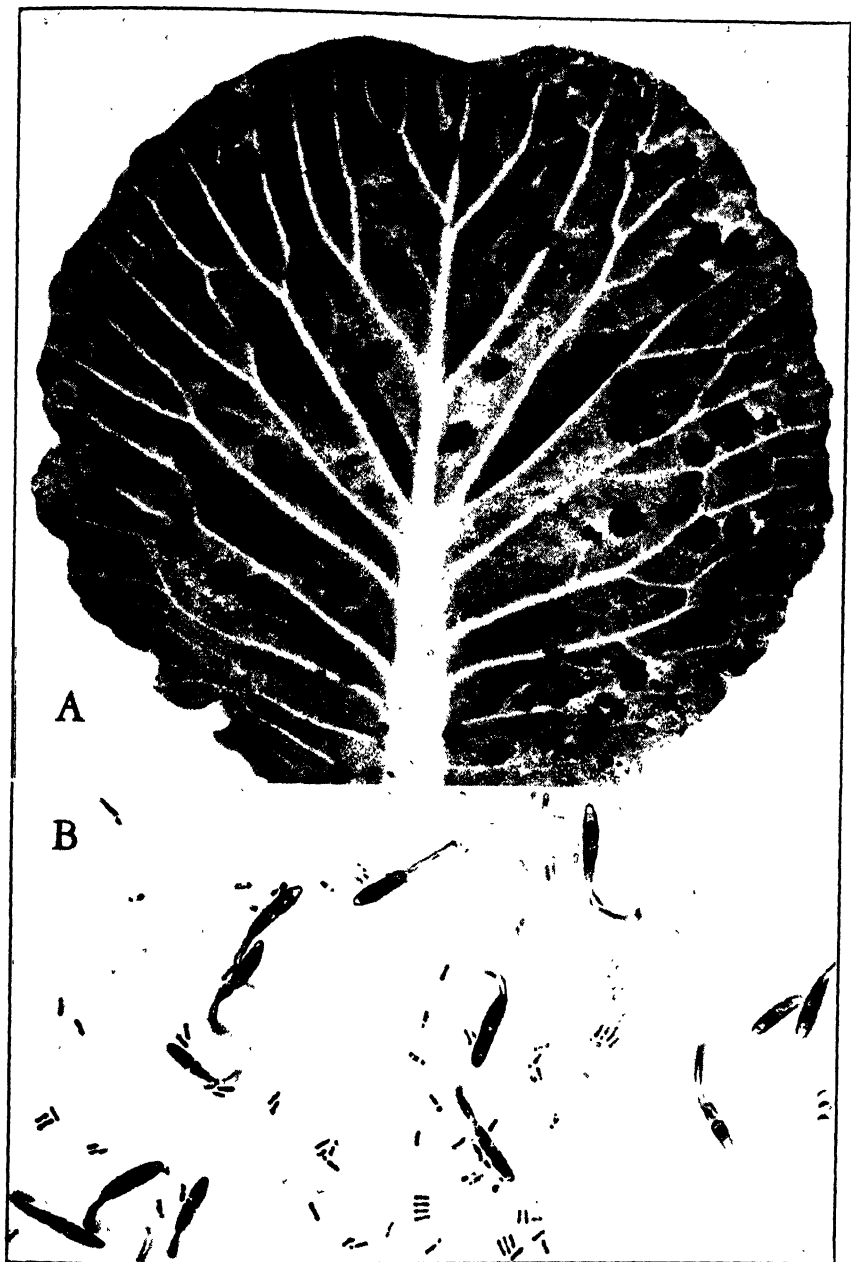


FIG. 1. A. Cabbage leaf naturally infected with ringspot. B. Germinated ascospores and ungerminated spermatia, in water, after 24 hours, 575 x.

Because of the failure of the so-called pycnospores to germinate or grow in or on various media, or to produce infection on cabbage under conditions favorable to ascospore growth and infection, and because of their character-

istic small size and appearance it is concluded that they are not pycnospores but spermatia. Correspondingly, the structures which produce them should be called spermogonia, not pycnidia. This interpretation would explain the lack of any tear-stain secondary infection on infected leaves in the field. It is, then, the concentric rings of spermogonia on the infected foliage which give ringspot its characteristic appearance. There are probably other diseases in which a spermatial stage similarly has been taken to be a conidial stage of the pathogen.

Recognition of the fact that *Mycosphaerella brassicicola* is a homothallic fungus possessing only one infectious (ascigerous) stage, spermogonia, and no conidia, indicates that dissemination is by means of wind not water. This bears directly upon a correct interpretation of the disease cycle of ringspot, and upon its control.—WILLIAM C. SNYDER, Division of Plant Pathology, University of California, Berkeley, California.

BOOK REVIEW

ANNA WEBER og CHR. STAPEL: *Bekaempelse af Haveplanternes sygdomme. Kortfattede, praktiske Anvisninger*. 11. forøgede og omarbejdede Udgave. Alm. Dansk Gartnerforening. 1944. (Control of diseases of garden crops. Short practical instructions. 11th increased and revised edition, by the Danish Horticultural Association, 1944.)

Everyone who ever has set about to write short practical instructions on the control of plant diseases knows the difficulties to be faced. One cannot expect an average farmer or gardener to know much about phytopathology, therefore one must give as detailed instructions as possible. On the other hand, these must be concise and not too much burdened with theory. The matter must be treated completely, but should not contain materials that are not essential for the practical man. It is not easy to find the right way between theory and practice.

Since this book has reached its 11th edition in 34 years, with more than 70,000 copies, and is known everywhere in Denmark as the "green book," one can presume that the authors have found this right way. And I dare say this little book is really an example of completeness, clearness, conciseness, and the right selection of subjects.

The book is divided into 4 parts. The first deals with diseases and insect pests occurring on many plants (32 pages, 62 alphabetically arranged paragraphs). In the second part the special injuries of the individual horticultural plants are dealt with in alphabetical order of the host plants (115 pages, 417 paragraphs). For each plant a first paragraph gives general instructions on sanitation, then the individual injuries follow in alphabetically arranged paragraphs. The third part contains a survey of the methods of control: cleaning, fertilizing, soil disinfection, seed dressing, dusting, spraying, fumigating, weed control, public control measures, import and export legislation, and compensation (14 pages, 11 paragraphs). In the fourth part (27 pages), finally, there follows an alphabetical survey of the fungicides and insecticides (41 paragraphs), the universal, Danish, English, and American measures and weights, some important addresses, and an alphabetical index.

The attention of phytopathologists all over the world is called not only to this individual book, but to all the practical books edited by workers in the Danish Plant Protection Service. There is no doubt that they are some of the best works dealing with the practical branch of our science. Unfortunately, the Danish language is little known, and this seems to have prevented their more universal use. But this should not be too serious an impediment; everyone who can read English and German will be able to make use of a Danish book, this language being somewhat intermediate between the two last named. And the little difficulty to be faced will be greatly rewarded in this case. Even those who may not read the Danish text will look at these books with great advantage, as they contain a great number of illustrations of an unsurpassed quality.

The following books also deal with the phytopathological and entomological sides of practical plant protection: S. ROSTRUP and M. THOMSEN: *Vort Landbrugs Skadedyr* (Pests of our agricultural crops), 4th edition, (Copenhagen, 1928; M. THOMSEN and P. BOVIEN: *Haveplanternes Skadedyr* (Pests of horticultural crops), Copenhagen, 1933; E. GRAM and P. BOVIEN: *Rodfrugternes Sygdomme og Skadedyr* (Diseases and pests of root crops), Copenhagen, 1943; CHR. STAPEL and P. BOVIEN: *Mark-Grøfgrøderne Sygdomme og Skadedyr* (Diseases and pests of seed-producing crops), Copenhagen, 1943. —H. BREMER, Central Institute of Plant Protection, Ankara, Turkey.

JAMES ROBERT WEIR
1881-1943

JOHN A. STEVENSON

James Robert Weir was born May 31, 1881, on a farm near Scottsburg, Indiana, and at his own request was buried there, following death from a cerebral hemorrhage on June 1, 1943. Dr. Weir was a descendant of Scottish immigrants, who came to the Ohio Valley from the Carolinas by way of Tennessee. As a farm boy he early became interested in the wild life about him, both plant and animal, and even prepared a manuscript treatise on "The natural history of Scott County" at the tender age of ten, although few books except an early edition of Wood's Botany and Dana's Geology were available to him. He graduated from the Scottsburg High School in 1900 and then attended the University of Indiana for several terms.

Wanderlust overtaking him, he worked for a time with forest survey parties in Alabama and Tennessee, taking part in some of the early reconnaissance surveys engaged in preparing forest working plans for the then Bureau of Forestry. Between seasons he matriculated at Purdue University as a special student, pursuing courses in botany under the noted botanists Stanley Coulter and J. C. Arthur. He finally returned to Indiana University where he remained until graduation in 1907 with an A.B. degree in botany.

He began his professional career as instructor in biology and chemistry at Culver Military Academy, serving in that capacity for two years. The spring of 1909 found him rambling through Central Europe botanizing and studying French and German. He entered the University of Munich in the fall, studying under Goebel in botany, Tubeuf in forest pathology, and Hertwig in zoology and receiving the Ph.D. degree in May, 1911. His doctoral dissertation dealt with the genus *Coprinus* in its morphological and physiological aspects.

Returning shortly thereafter to the United States, he almost immediately accepted a position as forest pathologist with the Office of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture. During the ensuing ten years, in cooperation with the United States Forest Service, he carried on pioneer investigations with forest tree diseases in the forests of the Northwest. His headquarters, originally at Missoula, Montana, were later transferred to Spokane, Washington, a number of years before his transfer to Washington, D. C., in 1921.

During this time—a fruitful period in his career—he published between 70 and 80 notes, bulletins, reports, and miscellaneous papers covering the results of his observations and researches on the problems encountered in his field. These papers, many of them in collaboration with his associate, E. E. Hubert, and some with the help of Annie Rathbun-Gravatt and Arthur S. Rhoads, covered a wide range of forest mycology and pathology, including

descriptions of new fungi found associated with tree diseases, records of forest tree disease surveys, studies of wood-rotting fungi, and life history studies of tree rusts and other forest fungi. Especially noteworthy were his extensive studies of the mistletoe parasites (the genus *Razoumofskya*)



JAMES ROBERT WEIR
1881-1943

of the forest trees of the Northwest. He gave particular attention to the development of methods of prevention and control of tree diseases as part of standard forest management practices. An indefatigable collector, he built up in the course of his work a comprehensive herbarium of fungi, including

not only those encountered in the forests through which he worked, but a valuable series obtained by exchange with mycologists throughout the world. This collection, notably rich in the Polyporaceae and other wood-inhabiting Hymenomycetes, now forms part of the mycological collections of the Bureau of Plant Industry.

With the closing of the Spokane laboratory in 1921, Dr. Weir came to Washington to write up that portion of his work remaining unpublished and was then transferred (1923) to assume charge of the mycological collections of the Bureau. His work with this unit was interrupted by extensive field trips.

During 1923 as pathologist, he was a member of the joint expedition of the Departments of Agriculture and Commerce to study the rubber production possibilities of the Amazon Valley of Brazil and Bolivia. During the course of this work a very large and important collection of fungi was made, including not only those concerned with diseases of *Hevea*, but all that he could find causing timber rot and disease of economic trees in the area covered. A comprehensive publication covering all known *Hevea* diseases and fungi resulted from this work. Following this expedition he travelled extensively in Argentina, Paraguay, Chile, and the West Indies, continuing intensive studies of diseases of tropical plants. Before resuming his work in Washington he spent two months in Cuba with the Sugar Experiment Station of the Tropical Plant Research Foundation where attention was given to sugar-cane-disease research and the possibilities of controlling marabú, a leguminous weed tree.

Early in 1927, the then newly organized Rubber Research Institute of Malaya invited Dr. Weir to join the staff as Head of the Department of Plant Pathology at Kuala Lumpur. Here he spent two years organizing work in his field. There followed a period of service as Director of the Plant Research Department of the Goodyear Rubber Plantations Co., Sumatra, Dutch East Indies. With broadened interests he established new silvicultural management practices for rubber lands, and studied improved tapping methods rather than confining his efforts to the purely pathological aspects of the crop as he had done, perforce, while working in Malaya. He took an active part in the Company's program of planting 40,000 acres of budded, high-yielding clones on its new Wingfoot Estate and investigated other tropical crops including tea, tobacco, coffee, sisal, and Manila hemp.

Returning to America late in 1932, planning to resume his studies in the taxonomy of the fungi, he was soon back in tropical harness with the Ford Motor Co., as an agricultural advisor of this concern on its Fordlandia and Belterra plantations along the Tapajoz River, a tributary of the Amazon. Here he found that extensive plantings had been made by transplanting wild seedlings without consideration for disease resistance or yield possibilities. His experience in the Orient had demonstrated clearly that whereas, "run of the mine" seedlings would yield only from 200 to 400 pounds per acre per year, selected clones with which he had worked would produce 700 pounds and even as high as 1500 pounds or more.

So within a few months he was back in the East Indies, buying selected plants of *Hevea* budded from the choicest high-yielding clones. Two thousand plants were finally secured, making a shipment of several tons in weight, all packed in specially built wardian cases and transshipped to Brazil with much care. He finally sailed up the Amazon and the Tapajoz with this material in the spring of 1934, some 58 years after Sir Henry Wickham had sailed down the same Rivers with his history-making collection of *Hevea* seed. It is of further interest to note that the Belterra plantation lies just across the Tapajoz River from the town of Boim, near which Wickham collected his seed. Some 1200 of Weir's plants survived in the nursery of Belterra to form the basis of subsequent large scale field plantings. And soon thereafter the International Rubber Committee in Singapore "locked the stable" by an order forbidding export of *Hevea* planting material in any form from territory under its control!

Following his service with the Ford Co., which terminated in March, 1938, Dr. Weir travelled extensively in Brazil and other countries of South and Central America with rubber-producing possibilities, studying *Hevea* cultivation and diseases and other complementary crop plants. During the years 1940-41 he was agricultural advisor to the Government of Venezuela with particular attention to increased rubber production. Death found him living quietly in retirement on the old home farm.

Dr. Weir was a member of many technical organizations during his active career in forest pathology, among which were the American Phytopathological Society, American Association for the Advancement of Science, Botanical Society of America, Society of American Foresters, Torrey Botanical Club, Ecological Society of America, and American Genetic Association.

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LYGUS BUG INJURY OF LIMA BEAN IN CALIFORNIA

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There occurs commonly in California a necrotic pitting of seeds of lima bean and blackeye cowpea which closely resembles the yeast spot reported in pathological literature except for the fact that no pathogen occurs in the lesions. Evidence is presented in this paper that the injury in California results from the feeding of *Lygus* bugs on the developing seed, substantiating an earlier note (2).

Large and baby lima beans and blackeye cowpeas in Ventura, Merced, San Joaquin, and Stanislaus counties and large limas in Orange and Los Angeles counties commonly have this trouble, and it also is said by W. W. Mackie to occur on cowpeas in Imperial County. It is referred to by growers and shippers as yeast spot, aphid spot, seed puncture, seed pitting, or dimpling. Nearly the entire dry large and baby lima crop of the United States (98.4 per cent in the period, 1934-43) is grown in California on about 150,000 acres of land in Ventura, Orange, Merced, San Joaquin, Stanislaus, San Diego, Los Angeles, and Santa Barbara counties. Green limas for fresh market and freezing are grown to a less extent in Los Angeles, Santa Clara, Orange, Lake, Stanislaus, and Ventura counties, occupying about 3,000 acres.

Losses from *Lygus* feeding result from: (a) shedding of blossoms and young pods; (b) discarding beans having conspicuous pits, or lowering of grade in fresh and frozen lima beans, particularly the latter, if such seeds are not removed; (c) cost of hand removal of pitted seeds from dry beans and from fresh limas to be used for quick-freezing; (d) occasional withering of the affected bean in an otherwise normal pod. The amount of actual loss sustained is uncertain because of the difficulty of comparison between fields and because the reduction in number or size of pods or beans may result in some size increase in the remaining beans. Also, plants with severe shedding of flowers and pods often continue to flower and may set a late crop of pods; in some cases such as in the Modesto area this late crop may mature, although tardily, but in other areas such as Ventura the bugs remain active in green fields and no late crop can be set. The injury frequently is so severe in local areas that the crop does not pay the cost of harvesting.

This seed pitting apparently was first observed in Michigan in 1895 on common bean (3) and definitely attributed to *Lygus* feeding. This association was reaffirmed in New York in 1919 (9) and was reported in Idaho in 1930 (20) where it also occurred on lima bean. The injury was said (1) to have occurred in California dried limas sold in Illinois in 1922 and was

¹ The writers acknowledge with pleasure the helpful suggestions of Dr. A. E. Michelbacher on some entomological phases of the study, and of Dr. P. G. Hoel on the statistical techniques used. Mr. A. A. Penkert, Oxnard, kindly supplied the plants analyzed in table 2.

attributed to yeast, apparently on the basis of symptoms. The pitting was first definitely associated with *Lygus* bug in California in 1944 by the writers. Fields with severe seed pitting and pod shedding frequently had large populations of these insects and were near recently cut alfalfa fields or other sources of infestation. Pods which contained pitted seed often had spots of sticky *Lygus* excrement. Examination of seed lesions failed to reveal *Nematospora* or other pathogens. It is known that *Lygus* bugs injure alfalfa (23), beet (10), and cotton (5) seeds, that they cause seed pitting of various beans (3, 9, 20) and that the insects feed on flowers and fruits or seeds. The demonstrated fact that these bugs are highly toxicogenic (21; 22, pp. 164-166) is consistent with the type of injury, a tissue breakdown rather than a mere puncture.

A similar type of injury to lima bean and blackeye cowpea was observed in Virginia in 1921 associated with the yeast, *Nematospora coryli* Pegl. (*N. phaseoli* Wingard), which was carried by the southern green stinkbug, *Nezara viridula* L. (*N. hilaris* Say) (29, 30). Such a relationship had first been shown by Nowell (16) in 1917 for internal boll disease of cotton. Yeast spot of lima bean has been reported in this country from Alabama (30), Illinois (1, 30), Maryland (8), Mississippi (8), North Carolina (8), Tennessee (30), Virginia (30), and West Virginia (14), as well as from Puerto Rico (8), Belgian Congo (24), Bermuda (26), and the West Indies (17). In addition, yeast spot has been reported in Oklahoma (13) on soybean and in South Africa (15) on kidney, mung, sugar, and soybeans and *Bauhinia golpini*. Other legumes reported as hosts include string bean (26), cowpea (17, 24, 29), bird's eye bean (29), and *Vigna catjang*, *V. unguiculata*, *Dolichos lablab*, *Canavallia gladiata*, *Crotalaria juncea*, *C. retusa*, and *Tephrosia*, *Indigofera*, and *Cassia* spp. (17). *Nematospora* spp. have been reported causing "stigmatomycosis" on fruits of a number of other hosts (tomato, cotton, pepper, orange, grapefruit, tangerine, pecan, *Datura metel*, *Asclepias curassavica*, pomegranate, coffee, *Sterculia platanifolia*, etc.) in association with several plant bugs. The only record of *N. coryli* in California in connection with an established plant disease apparently is that of Fawcett (6) on citrus, pomegranate, and cotton, although Schneider (19) found it on imported tomatoes here.

SYMPTOMS

The type of symptom produced on lima bean depends on the stage of pod and seed development at the time of *Lygus* feeding. If this occurs on blossoms or young pods they are quickly shed. Pods up to 2 inches or more in length commonly turn yellow, wither, and drop; these are referred to by growers as "buckskins" and attributed to high temperature. These symptoms resemble those which result from feeding of the green stinkbug, as illustrated by Underhill (25). Shedding may involve only a low percentage of the total pods set if only part of a field is infested or the infestation occurs tardily, or losses may be severe. In Ventura County in 1944 vining limas

in a 10-acre strip adjacent and parallel to a recently cut alfalfa field had, because of *Lygus* injury, set essentially no pods and were not threshed (11).

At the time of puncture of the pod a small hole may be visible from which sap oozes, and internally the tissue surrounding the puncture soon turns brown (20). External symptoms on such pods are lacking. Older pods have smooth, firm intumescences up to 10 mm. across and 2 mm. high on the inside surfaces (Fig. 1, A); such swellings have been reported from feeding of *Lygus* bugs on cotton stems (12) and bolls (5). These intumes-

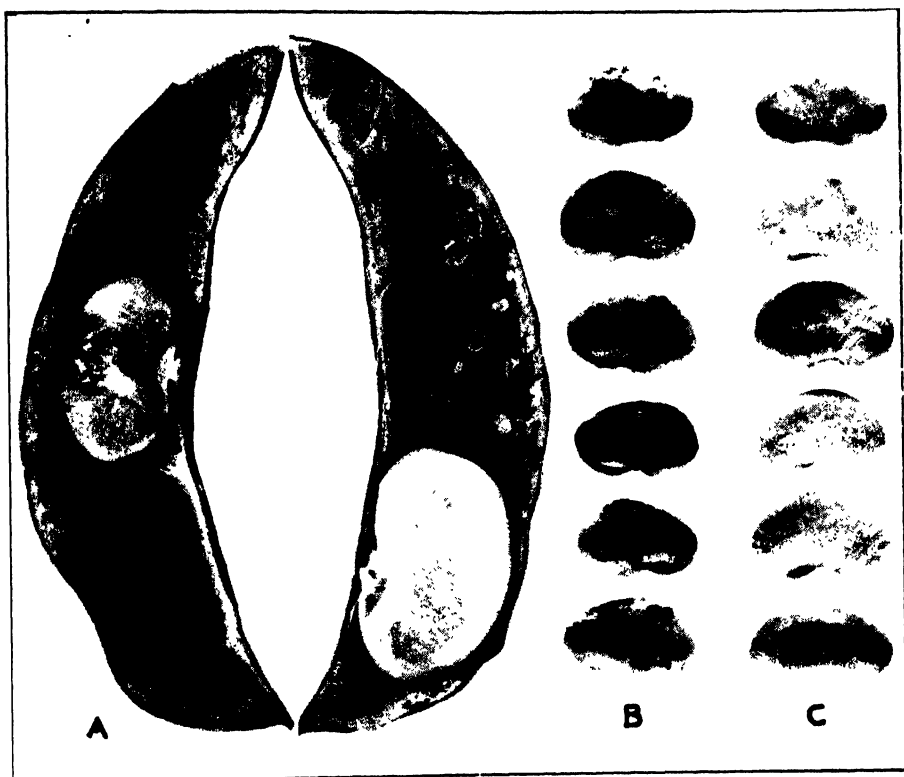


FIG. 1. *Lygus* injury of Ventura vining type lima bean produced by caging the bugs on plants grown in the greenhouse, 1945. A. Pod showing injuries and intumescences on inner surface of walls, and a pitted seed. B. Beans from pods on which *Lygus* were caged. C. Beans from pods on same plants caged without the insects.

cences frequently cause distortion by pressure against the developing seeds and usually are in contact with pitted areas of a seed. An occasional lack of coincidence is not surprising because (a) not all feeding punctures occur near seeds nor are all deep enough to reach a seed, and (b) changes in spatial relationship resulting from enlargement and crowding of seeds may destroy the coincidence of seed and pod punctures. The inside of a badly injured pod frequently has a frosty appearance from surface proliferation of parenchyma cells and the opposite walls of the pod sometimes fuse. As such pods mature a brown discoloration and tissue breakdown is visible on the inside

and, less definitely, on the outside. Examination of many pods has demonstrated the impossibility of accurately detecting either internal pod intumescences or seed pitting by external appearance of the pod, except by the infrequent incidence of slight swellings at the point of feeding.

In young seeds the initial injury may appear as a water-soaked area around a small hole; this soon shrivels to an irregular pit which probably enlarges as the seed grows. The seed may not attain normal size or may even shrivel, although the rest of the seeds in the pod are normal; other factors also cause such shriveling (Tables 1, 2). The pitted areas in full-

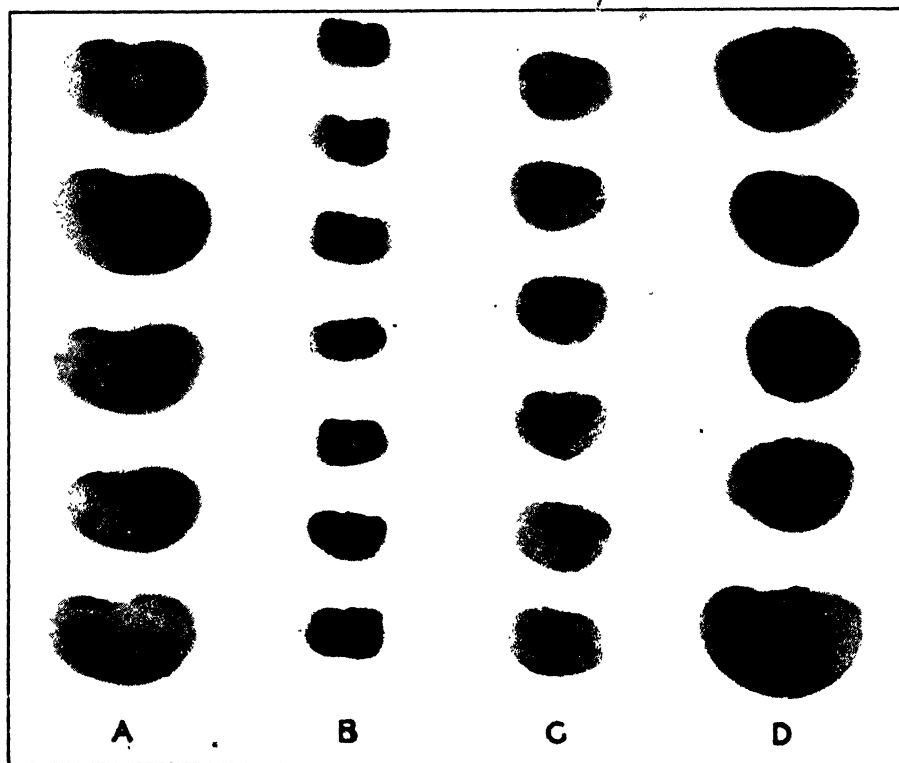


FIG. 2. *Lygus* pitting of field-grown lima bean and cowpea seed. 1944. A. Ventura vining type lima bean. B. Blackeye cowpea. C. Baby lima bean. D. Fordhook bush lima.

size beans vary from a tiny, sunken pin point, which may or may not rupture the testa, to large, irregular, crater-like, yellow or brown spots in the cotyledon over which the testa is destroyed. The tiny punctures without necrotic tissue apparently result from non-toxic feeding. Whether *Lygus* bugs vary in toxicity, as reported for other toxicogenic insects, is undetermined. The cavity is filled with a brown, granular, crumbly mass of necrotic cells and starch grains which is particularly evident before the seed dries. A similar necrosis from *Lygus* feeding has been reported on potato tubers (21, 22). Pits may occur at any point on the lima seed, singly or in such numbers that

coalescence results; as many as 52 punctures have been counted on a single seed. The testa may crack or even flake from dried beans, starting at such pits. Seed injury is shown in figures 1 and 2 and also in other papers (8, 20, 25, 29). Sometimes the integuments do not grow sufficiently to cover the uninjured cotyledons. This may represent (a) a gap left, by growth, when feeding killed a few cells of the very young integuments without injuring the cotyledons, or (b) a partial starvation of the integument tissue from injury to its vascular system in or near the funiculus. The second type could result from a feeding puncture which, had it occurred closer to the central vascular system of the funiculus, would cause the starvation or abortion of the entire seed.

Since the bugs are unable to feed on pods or seeds which have begun to toughen (20), and since feeding will cause shedding of young pods and abortion of young seeds, there is a relatively limited developmental period during which feeding will produce pitting of mature seeds. The percentage of seeds pitted may not, therefore, be high even in fields sustaining high total loss from the bugs. Shull (20) reported commercial fields of Great Northern beans with up to 4.09 per cent pitted seed and baby limas with up to 2.92 per cent.

Because Lygus bugs reach the highest population in mid- to late summer (20) the first pods set are relatively free of injury, but in cases of heavy later infestation essentially the total crop may be lost. Since the maximum damage occurs in the warmest season, the shedding of pods and flowers has been referred to as heat damage or, in the case of vining limas, as "steaming."

Microscopic examination of pitted areas has failed to reveal evidence of feeding tracks. This is in line with the absence of sheath material reported (12) in cotton on which various Miridae, including *Lygus oblineatus* (Say) (*L. pratensis* L.), had fed. Since the cells adjacent to the puncture usually are killed and shrunken, the continuity of sheath material would be destroyed.

SEED PITTING A RESULT OF LYGUS FEEDING

A number of circumstances in California differ sharply from those of the States reported to have *Nematospora* on lima beans. The yeast has been neither demonstrated in nor recovered from pitted lima bean seed grown here. The vector in the eastern area, the southern green stinkbug (*N. viridula*), apparently does not occur in the State. Beans are grown during the rainless summer months, a circumstance which practically eliminates seed infections by pathogens and would make for infrequent occurrence of *Nematospora*. Apparently these yeasts have only once (6) been reported causing a field disease in the State. Observations already presented caused the writers by August, 1944, to suspect Lygus bugs (*L. hesperus* Knight, *L. elisus* V.D., and perhaps others) as the true cause of the injury in California and subsequent work has confirmed this relationship.

Specimens of pitted blackeye cowpeas from Westley, California, large

limas from Ventura, and baby lima beans from Modesto, gathered in 1944 and submitted to W. E. Shull were found² to be identical with those produced by *Lygus* bugs in Idaho. The injury has not been observed on common bean in California. The Kentucky Wonder types of these beans with thick pod walls grown in Ventura, Santa Barbara, and Los Angeles counties would not be expected to show seed pitting, since Shull (20) found that it occurred only on thin-walled varieties.

A preliminary test with *Lygus* bugs collected near Oxnard in September, 1944, and caged on pods of lima beans growing in the field at West Los Angeles resulted in severe pod shedding after their feeding. As a consequence there was no seed pitting.

Insects collected near Ventura in September, 1945, were confined in cloth bags on clusters of flowers or young pods, and on older pods. The younger material was shed quickly in each case. When the older pods were examined after 7 days they contained typically pitted seed (Fig. 1) and the lesions were free from microorganisms.

TABLE 1.—*Incidence of seed pitting in lima beans grown in cloth cages with and without Lygus bugs. Insects added on July 31, Ventura County, 1945*

Location	Test condition	Lima bean seed				
		Total number	Average per pod	Per cent shriveled	Pitted	
					Number	Per cent
Oxnard (irrigated)	<i>Lygus</i>	688	1.86	30.2	36	5.2
	Check	596	1.83	32.4	0	0.0
Somis (unirrigated)	<i>Lygus</i>	1333	1.72	13.1	30	2.3
	Check ^a	1923	1.92	29.2	2	0.2
Camarillo (unirrigated)	<i>Lygus</i>	873	1.74	15.1	0	0.0
	Check	819	1.80	13.7	0	0.0

^a An uneaged equivalent area of an adjacent row had to be used and accounts for the pitted beans in this series.

Aster-cloth cages were placed over 30-foot sections of rows of vining type lima beans in several localities of Ventura County prior to seedling emergence. On July 31, 1945, approximately 200 *Lygus* bugs from alfalfa were placed in one cage in each location and the other was left as a check. The cages remained in place until the vines were mature and cut. Pods were harvested and the seed hand threshed. The incidence of seed pitting and shriveling in these trials is shown in table 1. In the Oxnard and Somis series there were 5.2 and 2.3 per cent of the seeds pitted in the *Lygus* cages, and 0 and 0.2 per cent in the checks. In the Camarillo test no pitting occurred in either cage, possibly because of some unfavorable environmental factor. The shriveling of the seeds in these tests obviously was not associated with *Lygus* injury, and the incidence of these aborted seeds is indicative of some physiological cause of much of this type of injury in the field. That *Lygus* can produce it occasionally is not doubted.

² Personal communication of May 31, 1945.

Additional data were obtained incidentally from an experimental plot near Oxnard set up by Holland in cooperation with the Division of Entomology, California Agricultural Experiment Station, Berkeley, to determine whether DDT would control Lygus. A 16.8-acre field of bush type lima beans was observed in early July, 1945, to be badly infested with Lygus bugs which had migrated from a large adjacent planting of seed beets. The field was divided into 3 parts: a 6-acre strip 60 rows wide next to the beets (check 1), the next 6-acre strip dusted with DDT (30 lbs. per acre of 4 per cent dust in sulphur) on July 28, the last 4.8-acre strip (check 2). On October 2, the date the field was cut, 100 plants were removed from the center row of each plot, 10 consecutive plants being taken without selection from each of 10 equally spaced points in the row. Data on these plants are given in table 2.

TABLE 2.—Data on lima bean plants from an area dusted once with DDT to reduce *Lygus* bug injury, and from the surrounding field. Ventura County, 1945

Items measured	Two check plots		DDT plot		Differences ^a
	n	Mean ^a	n	Mean ^a	
Green pods per plant	200	1.65 ± 0.16 ^b	100	3.01 ± 0.40	1.36 ± 0.43
Dry pods per plant	200	12.48 ± 0.46	100	19.90 ± 0.79	7.42 ± 0.91
Total seeds per plant	200	35.20 ± 1.29	100	61.60 ± 2.53	26.40 ± 2.84
Pitted seeds per plant	200	5.41 ± 0.41	100	3.04 ± 0.45	2.37 ± 0.61
Percentage seed unpitted	200	83.90 ± 1.13	100	93.10 ± 0.65	9.20 ± 1.30
Percentage seed shriveled	20	9.80 ± 1.37	10	15.20 ± 0.76	5.40 ± 1.57
Seeds per pod	20	2.56 ± 0.03	10	2.66 ± 0.04	0.10 ± 0.05 ^c
Total dry weight per 10 plants (oz.)	20	31.60 ± 1.71	10	40.30 ± 2.26	5.70 ± 2.83 ^c
Dry weight per 10 plants without pods (oz.)	20	16.20 ± 1.03	10	12.80 ± 0.77	3.40 ± 1.29
Dry weight of pods per 10 plants (oz.)	20	18.65 ± 0.91	10	27.80 ± 1.54	9.15 ± 1.79
Threshing seed yield per 10 plants (oz.)	20	14.00 ± 0.67	10	20.60 ± 1.11	6.60 ± 1.30
Threshing seed yield of pitted seed per 10 plants (oz.)	20	1.63 ± 0.20	10	0.90 ± 0.31	0.73 ± 0.37 ^c

^a Standard error given.

^b Difference between check plots significant; remaining differences not significant.

^c Difference not significant; remaining differences significant.

The only character showing a statistically significant difference between the two check plots was the number of green pods per plant. It is, therefore, justifiable to use a mean of the two plots for comparison with the dusted area. There were significantly more green pods, dry pods, and total seeds per plant, and a higher percentage of unpitted beans from the dusted than from the check areas. It might be argued that the given population of Lygus bugs had more pods on which to feed in the dusted than in the check areas, and that a smaller number of pitted seeds would therefore be expected. There was, however, no relationship between the number of pods per plant and the number of pitted seeds. The correlation coefficient for check 1 was 0.066 and was 0.074 for check 2; the correlation ratio for the dusted plot

was 0.111. This lack of correlation apparently resulted from the mobility of the insect. There was reduction in seed pitting and an increase in pod set, each independent of the other. There was a higher percentage of shriveled seeds in the dusted than in the check plots; this again suggests that *Lygus* bugs are not primarily responsible for seed shriveling.

The dusted plants were smaller than those from the checks but had a greater weight of pods, giving a total dry plant weight which was approximately equal. The weight of seed per 10 plants, cleaned so as to approximate field threshing, was likewise greater in the dusted than check areas; this apparently resulted mostly from increased pod set but somewhat from a slightly larger number of seeds per pod. The field-threshed yield was 20.31 bags per acre from the checks and 24.0 from the dusted area. A random sample of 650-800 beans was gathered directly from the threshing machine as it moved through each plot. These samples had 22.71 beans per ounce in the checks and 24.74 in the dusted plot. A test in another field yielded 21.06 and 23.0 bags per acre from the check and DDT plots, respectively, and these had 29.83 and 30.17 beans per ounce. The data indicate that there was little or no decrease in seed weight because of increased numbers.

One hundred green pods were gathered from one row of each plot on September 24 and examined carefully for *Lygus* damage. Check 1, check 2, and the dusted plot, respectively, had 84, 85, and 34 per cent of the pods with internal symptoms of *Lygus* feeding. The percentages of seed pitted were 53.4, 34.4, and 15.6, and the average number of seeds per pod were 3.11, 3.23, and 3.52, respectively. These data on green pods are essentially in line with those on the total crop, except that a much higher percentage of pitting was evident. Since the *Lygus* populations are highest late in the season, it seems reasonable that late setting pods would have more pitting.

These data may be interpreted as further evidence that *Lygus* bugs cause pod and blossom shed and seed pitting, and that control of the insects will reduce the amount of injury.

CONTROL

Since the reduction of *Lygus* injury is largely a matter of insect control, detailed methods are outside the scope of this paper. However, it may be mentioned that DDT has been found by several workers to be highly effective against these insects on various hosts. Also, field evidence suggests that the planting of lima beans next to a favored perennial host of the insect (*e.g.*, alfalfa, seed beets) is likely to lead to heavy losses, and should, therefore, be avoided.

DISCUSSION

The seed pitting of lima bean and cowpea in California is due to the feeding of *Lygus* bugs, the toxin secreted apparently causing the death of a considerable number of cells around the puncture. Because symptoms of the *Lygus* injury in California and Idaho specimens were identical with those of yeast spot of lima bean from Virginia, the difficulty of identification of the trouble solely on the basis of symptoms becomes apparent.

The writers have had no direct experience with the yeast-spot disease of the eastern states and cannot, therefore, evaluate that situation. The published record, however, presents some features which require clarification. It seems possible that the pitting attributed to yeast in certain areas may involve also an effect of insect toxins. Specimens of yeast spot of lima bean from Virginia³ proved to be identical in appearance with California samples, but microscopic examination and isolation attempts showed that numerous lesions had no *Nematospora*, although it did occur on some seeds. The only tests which have been reported (30) on transmission of the yeast to lima bean by the southern green stinkbug were made by transferring the insects from previously infected bean plants, and no results with uncontaminated bugs have been reported. The possibility seems not to have been excluded that a toxin introduced by the bugs might have caused damage, sometimes augmented by yeast infection. In fact, the reported rapid abscission of flowers and pods is suggestive of such a toxin rather than "the green stinkbug and the disease that accompanies it," as has been suggested (25). This shedding is not attributed by others to the yeast and it seems probable that it results from a toxin introduced by the insect, with or without yeast. Underhill (25) reported injury to lima bean from the direct feeding of the plant bug and infers that this may induce blossom fall. That blossom and pod fall result from *Lygus* feeding is clearly established (5, 20, 23) and the injury to lima beans in California is typical.

Several investigators (6, 8, 16, 27, 30, and others) have been unable to recover *Nematospora* from plant bugs supposedly carrying it. A few (7, 14, 15) succeeded in recovering the yeast from the bugs and found that transmission was purely mechanical, the insect placing the pathogen in a favorable substrate. The apparent difficulty of recovering the fungus from the insect, and the fact that not all lesions of lima bean, even in yeast spot regions, contain *Nematospora*, suggest that the yeast may be accessory to some other factor, perhaps an insect toxin. Because the southern green stinkbug is prevalent in both Virginia and West Virginia, but rarely carries *Nematospora* in the latter State, Leach and Clulo (14) concluded "that the association of the disease with the stinkbugs is not a constant one, even though the disease may depend upon the insect for inoculation." If no pitting occurs in West Virginia in the absence of the yeast, this might conceivably indicate the absence of toxicogenic strains of the southern green stinkbug or other insect, or might indicate the existence of ecological factors unfavorable to toxin production by them.

Inoculations with the yeast on uninjured lima bean pods were reported to be unsuccessful, but by puncturing the seed with a fine needle infections resulted (29, 30). That the yeast is pathogenic to immature lima bean seed under these conditions is not questioned. The pitting of lima bean in various areas, although similar in symptoms, possibly may result from such different agencies as toxicogenic insects and *Nematospora*. Such a complex

³ Supplied to W. W. Mackie through the courtesy of Dr. S. A. Wingard.

is not unprecedented. The kernel spot of pecan was attributed first to the feeding of *Nezara viridula* (4, 30), later to the *Nematospora* which they carried (28), and more recently (18) to injury by the bug itself. It is not implied that *Lygus* bugs are involved in pitting of lima beans in all areas nor that this is the sole insect involved in the injury in California.

SUMMARY

Large and baby lima beans grown in California for dry food, seed, the fresh market, and freezing commonly have a necrotic pitting of the seed which closely resembles the described yeast-spot disease, except for the absence of any pathogen. In one field test 16.10 per cent of the seed was so pitted, with as many as 52 punctures per seed. Similar damage occurs on blackeye cowpea, but has not been observed on common bean in California.

Field and greenhouse tests with *Lygus* bugs (*L. hesperus*, *L. clisus*, and perhaps others) caged on lima beans have demonstrated that the pits result from toxic feeding of the insects on the developing fruiting structures. Such *Lygus* injury and yeast spot cannot be distinguished on the basis of symptoms alone. The situation in other regions having this type of injury of lima bean should be reexamined to evaluate the factors (*e.g.*, insect toxins) of the complex.

The insects also cause, by toxic-feeding, a shedding of blossoms and of pods up to 2 inches long; this effect usually produces the most important loss from the bugs in California, amounting in small areas to the total crop. Reduction of grade and the cost of hand sorting of the beans also result from the seed pitting. There is some shriveling of young seed from *Lygus* feeding, but other undetermined factors appear to be even more important in causing this damage. Reduction of *Lygus* injury by one application of DDT dust resulted in significant increases in number of green and dry pods, number and weight of beans, and percentage of unpitted seeds. There was a decrease of dry plant weight exclusive of pods, but this was essentially balanced by the increased dry pod weight.

Injury tends to be worst in fields adjacent to established, perennial, favored hosts of the insect, such as seed beets and alfalfa. In some areas the insects may be present for a relatively short time on lima beans, injuring only a small part of the field and part of the pod set; in other areas the bugs remain active on the limas throughout much of the season, injuring most of the pods in whole fields.

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BUD, BLOSSOM, AND POD DROP OF CANNING STRING BEANS REDUCED BY PLANT HORMONES¹

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INTRODUCTION

A reduction in yield of canning string beans in northwestern Wisconsin has been experienced in recent years because of excessive drop of buds, blossoms, and small beans. Such losses have been greater in some years than in others.

Two factors have appeared associated with this blossom drop, *i.e.*, (1) hot, dry weather and (2) insects. Tarnished plant bugs, *Lygus oblineatus* (Say), and potato leafhoppers, *Empoasca fabae* (Harris), were present in sufficiently abundant numbers to warrant some control measure.

Insecticides have been used in the past to control both plant bugs and leafhoppers. Since Osborn (9) reported the use of pyrethrum to control the potato leafhopper, this insecticide has been used extensively. Recently pyrethrum and derris dusts appeared effective (10) in control of the tarnished plant bug.

Plant hormones, especially α -naphthalene acetic acid, which prevents the formation of abscission layers causing pre-harvest drop of apples (5, 6), seemed promising for preventing drop of blossoms and small beans. No reports have been found where such substances have been used for counter-acting insect injury to plants.

This paper presents the results of studies with plant hormones (1) to prevent drop of blossoms and small beans caused by insects or by weather and thus (2) to increase the yield of string beans. Preliminary reports (1, 2) have appeared earlier.

MATERIALS AND METHODS

Greenhouse Trials. In the greenhouse, Brittle (Round Pod Kidney) Bush Wax bean seeds were germinated in moist sand. When the primary leaves had begun to unfold, the vigorous healthy plants were transplanted individually into 6-inch pots containing a mixture of 1 part sand to 2 parts compost. In some cases the plants were used when they had their first trifoliate leaves but not their second, while in others, the plants were beginning to blossom or were in full blossom with small beans.

The plant hormones are designated in tables of results by the following abbreviations. These hormones were prepared in dusts containing from

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10 to 640 p.p.m. (parts per million) by mixing the chemically pure substances with Pyrax ABB:²

- 2B3NB = 2-bromo-3-nitro benzoic acid
- 2C5NB = 2-chloro-5-nitro benzoic acid
- PCIP = p-chlorophenoxy acetic acid
- DPA = 2,4-dichlorophenoxy acetic acid
- DPB = α -(2,4-dichlorophenoxy)-*n*-butyric acid
- IA = indole-3-acetic acid
- IB = (indole-3)-*n*-butyric acid
- LA = levulinic acid
- NA = α -naphthalene acetic acid
- BNA = β -naphthoxy acetic acid
- ANA = α -naphthyl acetamide
- PA = phenoxy acetamide
- PAA = phenoxy acetic acid
- P = Pyrax ABB
- TIB = 2,3,5-triiodobenzoic acid

The various concentrations of specific hormones were made from an initially prepared stock concentrate. From this, desired dilutions were prepared by adding Pyrax and further mixing in a ball mill.

A plant was dusted by placing it in a cylindrical chamber with a capacity of about 82 liters. A one-half gram sample of dust was expelled directly downward on the plant by compressed air, at 18 to 20 pounds' pressure, through a dust gun (3). The plant was exposed to the cloud of dust in the chamber for 90 seconds. Pyrax was used to dust check plants.

Since illuminating gas causes abscission of plant parts (4), it was employed in a partially gas-filled chamber to induce abscission of bean leaves and petioles. Whatever may be the relation between gas and insect feeding, both stimulate the formation of abscission layers. With gas one can easily regulate the incidence of dropping of leaves, buds, and pods. Thus it provided an easy technique for eliminating the less promising chemicals.

The illuminating gas used had the following components: illuminants (ethylene, propylene, and other hydrocarbon fractions), 8.8; carbon dioxide, 2.6; carbon monoxide, 31.6; hydrogen, 41.3; methane, 9.2; ethane, 1.0; nitrogen, 5.0; and oxygen, 0.5 per cent.

With gas treatment experiments, individually potted plants were employed when the first trifoliate leaves were well developed, but the second ones were not. A series of 6 or 8 plants was dusted. Best results were obtained when the soil about the plants was kept moderately moist. Excessive moisture or dryness was unfavorable for duplication of results. Watering immediately before the test provided a source of error.

Fifteen to 20 minutes after the plants had been dusted, they were placed

² Pyrax ABB is the symbol used to designate an inert material commonly employed in preparing insecticidal dusts.

in a circle inside a double glass-walled temperature chamber with a capacity of about 465 liters and a temperature of 84° to 86° F. A flask was filled by water displacement with about 3.45 liters of illuminating gas, and it was opened in the middle of the chamber. A small electric fan running slowly circulated the gas and air mixture of about 1:135. This concentration was hardly enough to have an odor.

All plants were removed from the gas chamber to the greenhouse after 24 to 26 hours. For a week readings were made of the number of abscissions of the various parts of each plant. Seven abscissions were possible in each plant; *i.e.*, the 2 primary leaf blades, the 2 petioles of the primary leaves, and each of the 3 leaflets of the first trifoliate.

Abscission by insects was induced by caging adult tarnished plant bugs on bean plants, which either were beginning to blossom or were in full blossom with small beans. Cages varied in type, including screen cages with a capacity of 425 and 100 liters, respectively, in which were placed dusted, potted bean plants infested with insects. Other small screen cylinders (20 by 40 mm.) confined the insects on plant parts, such as blossoms, beans, and portions of stems bearing flower clusters. Either cotton or Scotch tape was used to enclose the ends. Small paper sacks were also used.

Field Tests in 1942. In the field the problem of blossom and bean drop of canning beans was studied during the summer of 1942. The experimental plots located at Earl and Cumberland, Wisconsin, were planted to Brittle (Round Pod Kidney) Bush Wax beans and Idaho Refugee Bush Green beans, respectively.

Plots at both locations were identical, with separate dust and spray plots at each. All treatments were placed by chance in each of 4 replications. One treatment in each replicate consisted of four 15-foot rows of beans, making a total of 240 feet of row. A single buffer row was left between each unit, and a 2-foot buffer space separated the ends of units.

The following materials were employed in the dust and spray formulae: A commercial preparation (App-L-Set) containing 16 grams of α -naphthalene acetic acid per pound of talc was used in dusts. A commercial preparation (Parmone) containing 16 grams of α -naphthalene acetic acid per pint of alcoholic solvent was used in sprays. Powdered cubé insecticide containing 4.6 per cent rotenone was employed in dusts and sprays. Powdered pyrethrum insecticide containing 2 per cent pyrethrins was used in dusts and sprays. A spreading agent (Grasselli) was used in sprays. Pyrax ABB was the diluent for the dusts.

From these materials, the following were prepared: Dusts— α -naphthalene acetic acid, 140 p.p.m. and 70 p.p.m.; and α -naphthalene acetic acid, 140 p.p.m., plus pyrethrins 0.2 per cent and 0.5 per cent rotenone. Sprays— γ -naphthalene acetic acid, 7½ p.p.m., 5 p.p.m., and 2½ p.p.m.; and α -naphthalene acetic acid, 5 p.p.m., plus pyrethrins 0.2 per cent and 0.5 per cent rotenone.

The dusts were applied with a hand-operated rotary-type duster with

2 outlets directed upon 1 row. The sprayer was a Hudson barrel type with which a pressure of about 125 pounds was maintained. Two nozzles were directed on a single row. Dusts were applied at approximately 30 pounds per acre, and sprays were applied at about 100 gallons per acre.

Two applications of dusts and sprays were made to all the plots of beans. The first application to wax beans was made July 17 between 7 and 9 p.m., at which time there was no wind. It was very humid, and the temperature was 85° F. Lower blossoms on the plants were well opened, and buds on upper stems were closed but well developed. A heavy rain fell 24 hours after application. A second application was made July 24 between 7 and 9 p.m. It was rather humid, there was very little wind, and the temperature was 72° F. Small beans and well-developed blossoms were on the plants. Rain fell 8 hours after application.

Since Refugee is a later variety, dust and spray applications were made later in the season. The first application was made July 22 between 11 a.m. and 1 p.m. It was rather humid, there was a light wind, and the temperature was 70° F. About one-half of the blossoms was fully developed. Rain fell 6 hours after application. A second application was made July 30 between 7 and 9 p.m. Weather conditions were the same as for the first application. Small beans and well-developed blossoms were on the plants.

Five pickings of beans were made from each plot. To attain more uniformity in picking, each of four pickers harvested one replication of each treatment and check. The yield from each replicate was placed in separate bags, labeled, weighed, and graded by a mechanical grader.³

Some studies were made to determine the extent of damage caused by the tarnished plant bug and the potato leafhopper on wax bean plants. Observations were made of injury caused by these insects on plants dusted with α -naphthalene acetic acid at 70 p.p.m. as compared with insect-infested undusted plants. Wire cages of about 100 liters space confined the insects on the bean plants.

Field Tests in 1943 Five hormones, which had appeared best in the gas chamber trials, were chosen for field tests. Pyrax ABB was used as the dispersing agent for the hormone dusts.

Dusts for wax beans were employed at concentrations of 40, 80, and 160 p.p.m. with each of 4 hormones, *i.e.*, α -naphthalene acetic acid, levulinic acid, p-chlorophenoxy acetic acid, and 2,3,5-triiodobenzoic acid. At 20, 40, and 80 p.p.m. alone, 2,4-dichlorophenoxy acetic acid was used, and at these same concentrations containing 0.2 per cent pyrethrins and 0.5 per cent rotenone. Another dust contained only 0.2 per cent pyrethrins and 0.5 per cent rotenone.

Since α -naphthalene acetic acid did not appear effective on Refugee green beans in 1942, it was decided to try 2,4-dichlorophenoxy acetic acid at 20, 40, and 80 p.p.m. To similar preparations, 0.2 per cent pyrethrins and 0.5 per cent rotenone were added. A formula containing only 0.2 per cent pyrethrins and 0.5 per cent rotenone was also employed.

³ Located at the Stokely Canning Factory, Cumberland, Wisconsin.

Sprays for wax beans were employed at concentrations of 40, 80, and 160 p.p.m. of α -naphthalene acetic acid alone, and with 0.2 per cent pyrethrins and 0.5 per cent rotenone.

Wax bean dust plots, one each at Earl and Rice Lake, Wisconsin, consisted of 72 units with suitable buffers. Each unit consisted of 4 rows of beans, each row being 17 feet long. Each plot involved 20 treatments, replicated 3 times, and 12 checks.

Refugee green beans were dusted at Earl in a plot having the same arrangement as the spray plots there.

Wax bean spray plots were located at Earl and Rice Lake, Wisconsin.

TABLE 1.—*Summary of conditions when the field treatments were made in 1943*

Place, kind of bean, treatment, date	Applications made between	Weather ^a			Bean plant development
		Dew	Wind	Tempera- ture range	
	A.M.			° F.	
Earl, Wis.					
Wax beans					
Dust plot					
July 18	6: 30– 9: 00	Heavy	None	60–80	Blossoms
28	6: 30– 9: 00	Heavy	None	68–80	First picking completed
Spray plot					
July 19	10: 00–12: 00	Heavy	Light	82–82	Blossoms
27	8: 00–10: 00	Heavy	Light	80–80	Ready for first picking
Refugee beans					
Dust plot					
Aug. 1	9: 00–10: 00	Moderate	Light	84–84	Blossoms
8	6: 00– 8: 00	Heavy	None	68–70	Blossoms and 1 inch beans
Rice Lake, Wis.					
Wax beans					
Dust plot					
Aug. 1	6: 00– 8: 00	Heavy	None	68–68	Blossoms
7	6: 00– 8: 00	Heavy	None	68–68	Blossoms and 3 inch beans
Spray plot					
July 30	10: 00–12: 00	None	Light	82–82	Blossoms
Aug. 7	10: 00–12: 00	None	Light	80–80	Blossoms and 3-inch beans

^a There was no rain for several days after any treatment.

They consisted of 48 blocks equivalent in size to those of the dusted plots. Each treatment, as well as the untreated check, was replicated 6 times.

Demonstration plots were dusted on July 18 and 28, 1943, at Earl, Wisconsin, and on July 20 and 29, 1943, at Barron, Wisconsin. A dust composed of α -naphthalene acetic acid at 80 p.p.m. was applied to three 100-foot rows of beans. Parallel to these treated rows were 3 untreated rows of the same length.

Methods and equipment used in dusting, spraying, and recording yields and grades of the various treated and untreated beans were the same as employed in 1942.

A summary is presented in table 1 of the weather conditions at the time of the application of the dusts and sprays in the replicated plots.

Studies were made of damage to wax beans by the tarnished plant bug, the potato leafhopper, and the six-spotted leafhopper, *Macrostelus divisus* (Uhler). Trials were also made to prevent insect damage by dusting plants with α -naphthalene acetic acid at 80 p.p.m. Cages of about 100 liters capacity were used to retain insects of a particular species on the bean plants for observations.

EXPERIMENTAL RESULTS

Hormones Tested Against Gas-Induced Abscissions. Numerous exploratory trials were made with α -naphthalene acetic acid dusted on wax bean plants. Various constant temperatures and concentrations of illuminating

TABLE 2.—*Summary of evaluations by gas treatment of several plant hormones for prevention of abscission layer formation*

Hormones	Average abscissions ^a per plant from			Abscissions with hormones compared to those with Pyrax ^b	
	Hormones at 20 p.p.m.	Pyrax	Parallel NA at 10 p.p.m.	Hormones at 20 p.p.m.	Parallel NA at 10 p.p.m.
	No.	No.	No.	Per cent	Per cent
DPA	0.2	4.7	0.0	5	0
PCIP	2.7	6.2	1.5	44	24
DPB	1.5	3.0	0.0	50	0
IA	2.7	4.7	0.2	58	5
205NB	1.7	3.0	0.0	58	0
ANA	2.2	3.0	0.0	75	0
IB	4.5	5.7	0.7	78	13
PA	4.0	4.7	0.2	84	5
IA	5.0	5.7	0.7	87	13
TIB	5.7	6.2	1.5	92	24
BNA	6.0	6.5	4.0	92	61
213NB	3.2	3.0	0.0	108	0
PAA	5.2	4.7	0.0	111	0

^a Seven abscissions were possible on each plant.

^b Secured from dividing the number of abscissions with the hormone by those with Pyrax.

gas were used. It was found that a dust of α -naphthalene acetic acid at 10 p.p.m. with Pyrax prevented a great deal of abscission of the bean leaves and petioles after exposure for a day at 84° to 86° F. in 1 part of gas to 135 parts of air.

Comparisons of relative efficiency of these hormones in preventing abscissions were based on the use of Pyrax alone, α -naphthalene acetic acid at 10 p.p.m., and 2 other hormones at 20 p.p.m. Each experiment, performed twice, employed 8 plants, 2 for each of the 4 dusts to be used.

When the number of abscissions was recorded, notes were taken also of epinasty that developed in leaves and leaflets. Occasionally epinasty was only temporary. However, it was pronounced in those cases where abscission followed. Figure 1, A, shows successive stages of epinasty followed by abscission of the primary leaves.

The data presented in table 2, where both trials are averaged, indicate

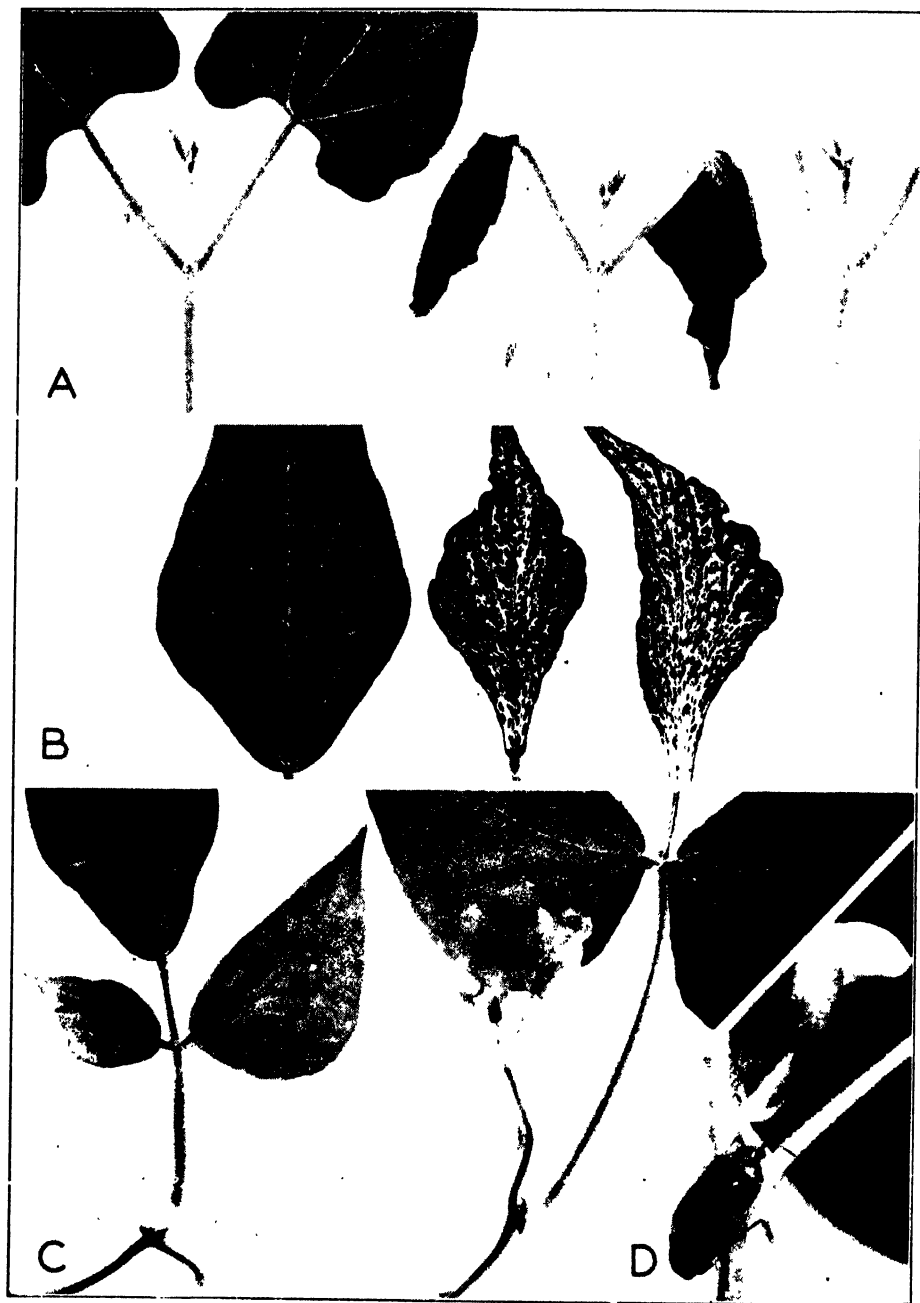


FIG. 1. Effects of illuminating gas, alpha-naphthalene acetic acid, and insects on kidney wax beans. A. Successive stages in leaf drop induced by illuminating gas. B. Left, untreated leaflet. Center and right, symptoms resembling mosaic induced by the hormone in dust form at 640 p.p.m. C. Left, *Lygus oblineatus* has damaged untreated leaflets and has induced blossom drop. Right, blossom drop was prevented with the hormone at 80 p.p.m. D. *L. oblineatus* feeding on pedicels. Approximately $\times 2$.

that α -naphthalene acetic acid (NA) at 10 p.p.m. is superior to the other hormones used at 20 p.p.m., except 2,4-dichlorophenoxy acetic acid (DPA). Among the materials tested, α -naphthalene acetic acid and 2,4-dichloro-

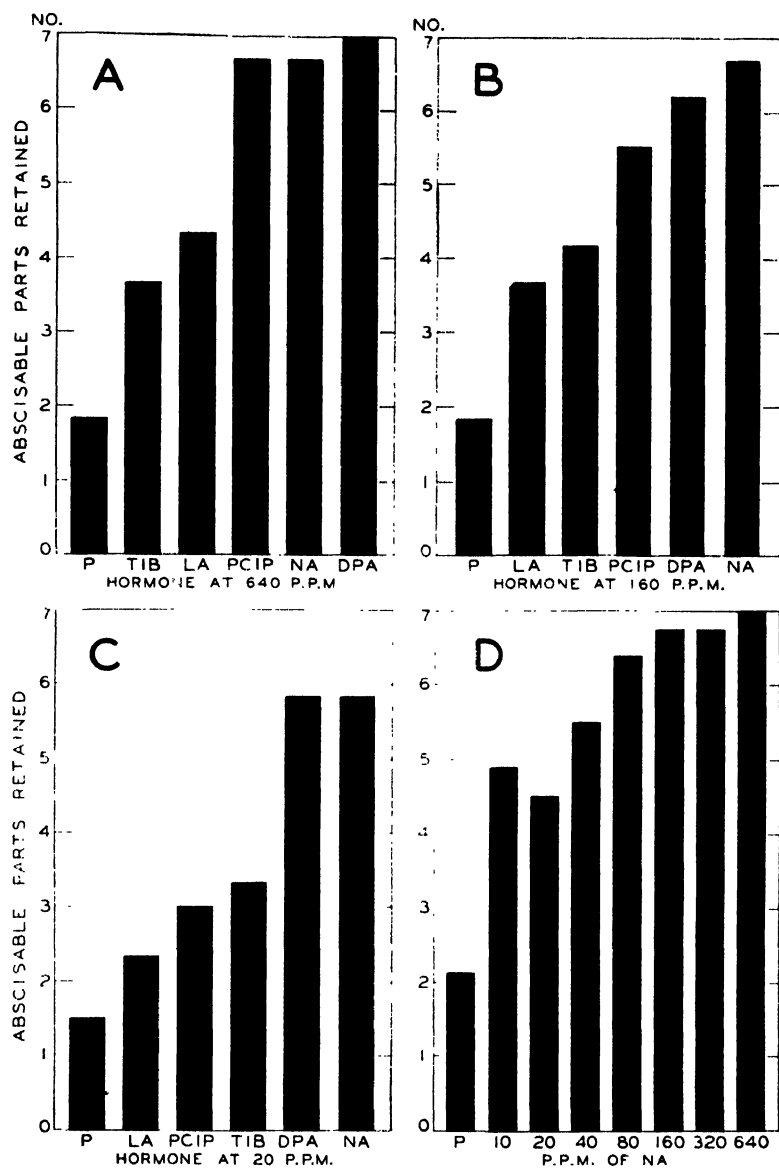


FIG. 2. Summary of retention of abscissable parts by bean plants after treatments, both with gas and with hormones of different kinds at various concentrations. Abbreviations are explained in the text.

phenoxy acetic acid were effective, while p-chlorophenoxy acetic acid (PCIP) and levulinic acid (LA) were moderately effective in preventing abscissions. Each of these materials and triiodobenzoic acid were then prepared in dusts

at 20, 160, and 640 p.p.m. Although data presented in table 2 show triiodobenzoic acid to be poor, it seemed worth testing at higher concentrations, because it was not a finely divided crystalline material, and thus may not have been mixed well at a low concentration.

Three experiments were made to compare the 5 hormones at the 3 specific concentrations designated. In each experiment there were 6 plants, 5 for comparison of hormones at one concentration, and 1 Pyrax check. Six replications of each experiment were made, and a change in position of the various treatments within the chamber was made for each successive replication. No evidence, however, indicated that such change in position was necessary.

Table 3 shows a comparison of the abscission²-prevention qualities of hormones at 640 p.p.m. Similar tables could be shown for the other 2 experiments, with the hormones at 160 p.p.m. and 20 p.p.m., but the data of all three are expressed graphically in figure 2, A, B, and C, which show the average number of abscissable parts retained by the plants. In the hormone comparisons at 160 p.p.m., the M.S.D. (minimum significant difference) at the 5 per cent level was 1.35 (mean), and 1.84 (mean) at the 1 per cent level. In the 20 p.p.m. comparisons, the M.S.D. was 1.33 (mean) at the 5 per cent level, and 1.82 (mean) at the 1 per cent level. All 5 hormones gave significant results at the 5 per cent level when used at concentrations of 640 and 160 p.p.m. At 20 p.p.m., only levulinic acid results were not considered significant. Most significant results were obtained with α -naphthalene acetic acid and 2,4-dichlorophenoxy acetic acid.

To obtain information as to the effectiveness of α -naphthalene acetic acid at several strengths in dust form, concentrations of 10, 20, 40, 80, 160, 320, and 640 p.p.m. were tested. Each of 7 bean plants was dusted with a different concentration of the hormone, and another plant was dusted with Pyrax to serve as a check. This group of 8 plants was subjected to gas in the chamber. The procedure was replicated 8 times, and a change in position of the various treatments within the chamber was made for each successive replication.

Figure 2, D, which indicates the average number of abscissable parts retained by plants, shows the ability of several α -naphthalene acetic acid concentrations to prevent abscission.

During the preceding experiments, it was observed that the higher concentrations of α -naphthalene acetic acid and 2,4-dichlorophenoxy acetic acid caused virus-like symptoms of vein-clearing, distortion, and dwarfing of bean plant leaflets. Since these observations were of plants which had been placed in the gas chamber, tests were made to produce such symptoms, if possible, on hormone-dusted plants permitted to grow in the greenhouse without being subjected to the gas chamber.

Each of 16 wax bean plants containing a single trifoliolate was dusted with a $\frac{1}{2}$ -gram sample of dust. Two plants received either α -naphthalene acetic acid at 10, 20, 40, 80, 160, 320, 640 p.p.m. or Pyrax. After treatment these plants were permitted to grow until all had trifoliate, at which stage observations were made for possible damage.

Plants dusted with α -naphthalene acetic acid at 640 p.p.m. showed vein-clearing, distortion, dwarfing, and a darker green color of the leaflets of the third to seventh, inclusive, trifoliates on 1 plant and third to eighth, inclusive, on the other (Fig. 1, B). The plants dusted with the 320 p.p.m. concentration were similarly, though slightly less, affected on the third to fifth, inclusive, trifoliates on 1 plant and fourth to eighth, inclusive, on the other. The 160 p.p.m. concentration produced a slight tendency toward these symptoms in the third to sixth, inclusive, trifoliates. The other concentrations of the hormone and Pyrax did not cause these symptoms.

Parallel experiments with 2,4-dichlorophenoxy acetic acid produced symptoms similar to those obtained with α -naphthalene acetic acid when the former was used at 640 and 320 p.p.m., but the 160 p.p.m. concentration did not produce the symptoms.

TABLE 3.—Comparison of abscission-prevention qualities of five hormones at 640 p.p.m.

Replicates	Abscissions					
	P	NA 640	LA 640	DPA 640	PCIP 640	TIB 640
1	5	0	3	0	2	6
2	4	2	1	0	0	4
3	5	0	4	0	0	5
4	4	0	0	0	0	2
5	6	0	4	0	0	1
6	7	0	4	0	0	2
Total	31	2	16	0	2	20
Average ^a	5.17	0.33	2.67	0.0	0.33	3.33

^a M.S.D. at 5 per cent level—1.50 (mean); at 1 per cent level—2.05 (mean).

Symptoms similar to those produced on wax beans occurred on Henderson Bush Lima beans when they were dusted with α -naphthalene acetic acid at the same concentrations. Each plant had developed 2 trifoliolate leaves at the time of dusting. The 640 p.p.m. hormone dust produced the symptoms on the succeeding 5 trifoliates of 1 plant, and on 6 of another. Slightly less evident were these symptoms on the succeeding 2 and 3 trifoliates of 2 plants dusted with the 320 p.p.m. concentration.

The tests show that these symptoms move upward into the developing trifoliates. These virus-like symptoms were similar to those reported by Zimmerman and Hitchcock (11), and doubtless were caused by the hormone and not by a virus. Limited trials on virus transmission were negative. In all of the experimental work carried out, no bean plants were employed which developed mosaic. Scarcely any of the plants grown for testing showed mosaic symptoms and in these occasional appearances none resembled the hormone effects.

Insect-induced Abscissions Prevented by Hormones. During the first week of July, 1942, a survey of canning bean plantings was made in Barron, Burnett, Polk, Rusk, Sawyer, and Washburn Counties of Wisconsin to deter-

mine the insect species most abundant on string beans. Species most noticeable were: potato leafhopper, *Empoasca fabae* (Harris)—six-spotted leafhopper, *Macrostelus divinus* (Uhler)—tarnished plant bug, *Lygus oblineatus* (Say)—rapid plant bug, *Adelphocoris rapidus* (Say)—alfalfa plant bug, *Adelphocoris lincolatus* (Goeze)—potato flea beetle, *Epitrix cucumeris*

TABLE 4.—Field and greenhouse trials with feeding by insects on entire plants in full bloom, and hormone treatments in relation to beans produced

Date	Insects		Feeding ^a time	Plants	Concen- tration NA	Total beans produced
	Species	Popu- lation				
		No.	Days	No.	P.p.m.	No.
<i>Field trials</i>						
July 15, '42	<i>Lygus oblineatus</i>	12	37	3	70	9
	do	12	37	3	0	7
	<i>Empoasca fabae</i>	30	37	3	70	12
	do	30	37	3	0	12
	None	0		3	0	15
July 12, '43	<i>L. oblineatus</i>	18	25	3	80	10
	do	18	25	3	80	9
	do	18	25	3	0	8
	<i>E. fabae</i>	40	25	3	80	12
	do	40	25	3	80	13
	do	40	25	3	0	13
	<i>Macrostelus divinus</i>	40	25	3	80	16
	do	40	25	3	80	16
	do	40	25	3	0	16
	None	0		3	0	14
	do	0		3	0	17
<i>Greenhouse trials</i>						
May 5, '43	<i>L. oblineatus</i> ^b	17	25	3	10	8
	do	0	25	3	0	6
	None	0		3	0	11
Nov. 30, '43	<i>L. oblineatus</i>	30	29	2	80	4
	do	0	29	2	0	1
	None	0		2	0	11
Oct. 11, '44	<i>L. oblineatus</i>	55	4	4	80	12
	do	55	4	4	0	10
	do	55	4	4	80	10
	do	55	4	4	0	7
	None	0		4	0	20
Nov. 18, '44	<i>L. oblineatus</i>	15	2	2	160	4
	do	15	2	2	0	4
Nov. 23, '44	<i>L. oblineatus</i>	45	8	3	80	7
	do	45	8	3	0	4

^a Insects could feed on the entire aerial parts of the plants.

^b The 17 insects were free to feed on all 6 plants. The brackets indicate a similar situation in other trials.

(Harris)—striped flea beetle, *Systema taciata* (Say)—spinach flea beetle, *Disonycha xanthomelaena* (Dalman)—spotted cucumber beetle, *Diabrotica duodecimpunctata* (F.)—striped cucumber beetle, *Diabrotica vittata* (F.)—bean leaf beetle, *Ceratoma trifurcata* (Forst.)—bean aphid, *Aphis rumicis* L.—leafhopper, *Polyamia inimicus* (Say).

The first 5 species were the most prevalent. They feed by sucking plant

juices, and the damage is usually not noticeable until considerable injury has been accomplished. The feeding of some plant bugs (Miridae) has been associated particularly with the blasting of blossoms. Such insects, especially the tarnished plant bug, rapid plant bug, and alfalfa plant bug, were usually found feeding on or near the blossoms and small beans (Fig. 1, D). The effect of such feeding appears in figure 1, C, where the plant at the left has lost buds and blossoms and has small and more or less distorted leaves in comparison with the hormone-dusted plant at the right.

To ascertain injury to wax bean plants caused by the 3 most abundant insects inhabiting the bean foliage in the field, *viz.*, potato leafhopper, six-spotted leafhopper, and tarnished plant bug, these insects were collected according to species and caged on healthy plants in the field and greenhouse. Incorporated in the trials were plants dusted with α -naphthalene acetic acid. Specific insects were caged also on these plants to determine if the hormone might control insect damage. Wire cages with a capacity of 100 liters were used in the field; and in the greenhouse, wire cages with a capacity of 425 liters.

Table 4 presents the data secured from field and greenhouse trials relating to reduced bean yields caused by some insects and the prevention of some of the damage by hormone dust treatments of the plants. Very few of the insects were alive at the end of the longer feeding periods.

These insect studies on entire plants indicated that *Macrostelus divinus* had no detrimental effect, that *Empoasca fabae* had some aside from hopper-burn, and that *Lygus oblineatus* had considerable. This effect of *L. oblineatus* appeared (Fig. 1, C) on the leaflets as reduction in size, wrinkling, and mottling. It appeared on the fruiting parts either as bud, blossom, or pod drop, or as malformation of pods retained, with resulting reduction in yield. Some of this damage was reduced by dusting the bean plants with α -naphthalene acetic acid.

To determine results of insect feeding in localized areas of the plants, small numbers of tarnished plant bugs were caged on wax bean blossom clusters by means of a small paper bag, or were caged on a stem from 1 to 2 inches below the basal attachments of blossom pedicels. At 24 to 48 hours preceding the placement of insects, some of the bean plants were dusted with α -naphthalene acetic acid and the others with Pyrax. After varying lengths of time, the insects were removed, and the ultimate production of mature beans was recorded. Reference is made to these trials in table 5.

The feeding of the tarnished plant bugs on wax bean blossoms will cause the blossoms and small beans to blast, thus a reduced yield of beans. This insect may also cause blossom and small bean blasting, and a resultant reduced yield by feeding on that part of a stem between 1 and 2 inches below the basal attachment of pedicels bearing blossoms. Bean plants were dusted with α -naphthalene acetic acid before these insects were allowed to feed, and the reduction in yield was not so great as with those untreated. Beans from untreated plants were more apt to be malformed and small.

TABLE 5.—*Greenhouse trials with feeding by a population of three *Lygus obtinatus* on flower parts, and hormone treatments in relation to beans produced*

Date, 1944	Feeding time	Bean plants		Concentra- tion NA	Beans produced
		Blossoms	Plants		
	Hrs.	No.	No.	P.p.m.	No.
<i>Insects feeding on blossoms^a</i>					
Nov. 16	27	6	2	160	2
	27	6	2	0	1
Nov. 16	75	9	3	80	5
	75	9	3	0	2
Nov. 23	120	9	3	80	3
	120	9	3	0	2
<i>Insects feeding on stems^b</i>					
Nov. 20	144	6	2	80	3
	144	6	2	0	1
Nov. 24	96	9	3	80	4
	96	9	3	0	2
Nov. 25	120	9	3	80	3
	120	9	3	0	1

^a Entire blossom cluster containing 3 blossoms.^b Stem supporting 3 pedicels, each bearing 1 blossom.

Bean Yields Increased in 1942 by Hormones. Yields of beans following application of α -naphthalene acetic acid dust at 140 p.p.m. in 1942 (see 1 and 2) are shown in detail in table 6 as an example of the procedure. The treated plants had an increase in yields of 18.4 per cent over the untreated. This figure is designated as + 18 in summary table 7, where the results appear from different concentrations and combinations, from two varieties, and from two methods of application. Applications to Round Pod Kidney Wax Beans of α -naphthalene acetic acid as a dust at either 70 or at 140 p.p.m. with

TABLE 6.—*Yields of Wax beans from representative hormone-treated and untreated blocks at Earl, Wisconsin, in 1942*

Treatment and picking date	Yield in replicates				Total yields
	1	2	3	4	
	Oz.	Oz.	Oz.	Oz.	Oz.
<i>Dust treatment—NA 140 p.p.m.</i>					
July 31	100	128	112	140	480
Aug. 6	168	176	164	196	704
Aug. 11	64	56	44	64	228
Aug. 17	54	48	48	40	200
Aug. 21	24	12	12	12	60
Total	420	420	380	452	1672 ^a
<i>No treatment (controls)</i>					
July 31	92	88	84	68	332
Aug. 6	124	176	168	144	612
Aug. 11	64	52	52	40	208
Aug. 17	56	52	40	52	200
Aug. 21	20	16	12	12	60
Total	356	384	356	316	1412

^a 18 per cent increase over untreated.

insecticides, respectively, gave increased yields of 17.8 and 15.6 per cent, which appear as 18 and 16 per cent in table 7. The addition of pyrethrum and cubé to the hormone dust at strengths employed did not significantly influence the yield, probably because they did not adequately control insects. None of the sprays was significantly beneficial, and some were harmful, possibly because the force of the spray knocked off some blossoms, and because the hormone was not present in a suitable form. Murneek, Wittwer, and Hemphill (8) have reported beneficial as well as detrimental results from spraying snap beans with hormones. As explained earlier, the strength of the treatments was rapidly reduced in every case by rains that came some hours after the treatment.

It is possible that α -naphthalene acetic acid may be specific for various bean varieties, similar to its varietal specificity in preventing pre-harvest

TABLE 7.—*Total yields of beans expressed in percentage increase or decrease over check from the dust and spray plots of Wax and Refugee beans*

Treatments	Percentage increase or decrease over check ^a	
	Wax beans	Refugee beans
<i>Dusts</i>		
(NA 140 p.p.m.)	+ 18	+ 8
(NA 70 p.p.m.)	+ 18	+ 9
(NA 140 p.p.m., pyrethrins 0.2 per cent + rotenone 0.5 per cent)	+ 16	+ 3
<i>Sprays</i>		
(NA 7½ p.p.m.)	+ 3	- 4
(NA 5 p.p.m.)	+ 4	- 11
(NA 2½ p.p.m.)	+ 1	+ 7
(NA 5 p.p.m., pyrethrins 0.2 per cent + rotenone 0.5 per cent)	+ 8	0

^a Untreated controls in Wax bean dust plots yielded 4805 pounds per acre, in Wax spray plots 5064 pounds, in Refugee dust plots 8737 pounds, and in Refugee spray plots 8465 pounds.

apple drop as reported by Hitchcock and Zimmerman (7). This may account for the small increase in yields of hormone-dusted Refugee beans. At the same time these beans are later and may not be subject either to the insects or hot weather that favor blossom drop.

These data did not show whether the increased yield in hormone-dusted plots was due to production of more beans or of larger beans. Consequently, in every case a sample of 100 ounces of beans from each replication of each treatment and from each check after each picking was passed through the canning factory mechanical grader. Records were kept of Grade A and Grade B beans, the former being the smaller, higher-grade beans.

The average percentage of Grade A beans was 51 for the check and 54, 55, and 55 for the respective dust treatments, which employed the hormone and which averaged about a 17 per cent greater yield than the check. Apparently a larger number of small beans occurred in the hormone-treated pick-

ings than in the untreated. It seems clear that the 17 per cent increased yield was due to more beans, rather than to larger beans. As is generally true with many plants, the more units of fruit there are produced, the smaller are the fruits. Smaller beans bring higher prices for canning. Since increased yields came from more beans of smaller size in comparison with the check, it follows that the treated plants retained more blossoms and beans than those untreated.

Bean Yields Increased in 1943 by Hormones. Due to the favorable results obtained with α -naphthalene acetic acid used as dust in 1942, laboratory, as well as field, trials were made in 1943. Employing the information gained from testing abscission-preventing substances⁸ under laboratory conditions with illuminating gas, it was possible to arrive at concentrations of

TABLE 8.—*Yields in ounces of Wax beans from α -naphthalene acetic acid dust plot at Earl, Wisconsin, in 1943*

Picking date	Beans from plants treated with α -naphthalene acetic acid dust at							
	40 p.p.m.				80 p.p.m.			
	Replicates				Replicates			
	1	2	3	Total	1	2	3	Total
	Oz.	Oz.	Oz.	Oz.	Oz.	Oz.	Oz.	Oz.
July 27	34	23	39	96	31	32	30	93
Aug. 1	96	108	116	320	113	86	116	315
Aug. 5	40	41	77	158	78	81	63	222
Aug. 9	44	57	77	178	44	57	84	185
Aug. 14	25	17	81	123	25	29	69	123
Aug. 25	49	31	60	140	33	46	34	113
Sept. 9	127	117	80	324	57	70	24	151
Total	415	394	530	1339	381	401	420	1202

* Total yield of untreated beans was 1073 ounces. With the hormone at 40 p.p.m., there was a change of +24 per cent, and at 80 p.p.m., +12 per cent, which figures appear with the results at Earl in table 9.

particular plant hormones most desirable for use in field trials. Because of the larger number of materials and concentrations employed, there were only 3 replications in the 1943 field plots.

Yields in ounces of beans picked from the various replicated experimental plots were recorded as before. A part of the detailed data secured is presented in table 8 as an example.

During this season various difficulties appeared for accurate record taking. The larger size of the plots and changing personnel made it impossible to have one picker take an entire replication as in the previous year. This accounts for at least a large part of the increased variability of results over those secured the previous year.

The results of these 1943 trials appear in summary form in table 9. An analysis of variance on the data indicated no significance at the 5 per cent point in the plot at Rice Lake. However, there was significance in results at Earl, and also in results from these 2 plots when taken together, for the

beneficial effect of α -naphthalene acetic acid at 40 p.p.m. and at 80 p.p.m. The detrimental effect of this acid at 160 p.p.m. in dust and at all concentrations in sprays was apparent. None of the other substances appeared particularly good or bad.

The poor showing from the sprays was caused in part by the mechanical damage from the high pressure (125 lb.) which knocked off many blossoms and small beans. This is apparent since the yields from beans sprayed with

TABLE 9.—*Summary of percentage increase (+) or decrease (–) in yields produced by Wax beans treated with hormones in 1943*

Substances used	Amount of hormone used	Results with wax beans at	
		Earl ^a	Rice Lake ^a
	<i>P.p.m.</i>	<i>Per cent</i>	<i>Per cent</i>
<i>Dusts</i>			
α -Naphthalene acetic acid	40	+ 24	+ 14
do	80	+ 12	+ 8
do	160	– 17	– 1
2,4-Dichlorophenoxy acetic acid (No. 1)	20	– 5	– 6
do	40	– 13	+ 8
do	80	+ 2	+ 1
2,4-Dichlorophenoxy acetic acid (No. 1) + pyrethrins 0.2 per cent + rotenone 0.5 per cent	20	– 10	– 1
do	40	– 4	– 1
do	80	+ 1	– 5
2,4-Dichlorophenoxy acetic acid (No. 2)	40	– 11	+ 9
2,3,5-Triiodobenzoic acid	40	– 9	+ 13
do	80	+ 10	– 10
do	160	– 1	+ 4
p-Chlorophenoxy acetic acid	40	– 5	0
do	80	+ 3	– 3
do	160	+ 7	– 10
Levulinic acid	40	+ 6	– 4
do	80	+ 3	+ 4
do	160	– 10	+ 1
Pyrethrins 0.2 per cent + rotenone 0.5 per cent	0	+ 3	0
Untreated controls	0	0	0
<i>Sprays</i>			
α -Naphthalene acetic acid	40	– 27	– 23
do	80	– 35	– 42
do	160	– 62	– 60
Pyrethrins 0.2 per cent + rotenone 0.5 per cent	0	– 29	– 14

^a Untreated controls in dust plot yielded 3540 pounds per acre at Earl and 5185 pounds per acre at Rice Lake.

pyrethrum and cubé, as well as those from beans sprayed with α -naphthalene acetic acid, were significantly less than from unsprayed beans. As the concentration of α -naphthalene acetic acid in sprays was increased, the yield of beans was decreased. Leaflets of plants sprayed with α -naphthalene acetic acid also showed the appearance of crinkle, curl, and mosaic and thus resembled virus symptoms.

The difference in results secured with α -naphthalene acetic acid at 160 p.p.m. in 1943 and at 140 p.p.m. in 1942 is not particularly surprising. The actual situation was that in every case in 1942 a rain followed the treatment

by some hours, which doubtless either greatly reduced the effective concentration on the plants or prevented it from acting long enough to be very damaging.

The results in demonstration trials, described earlier, appear in table 10 which lists the yields of the Round Pod Kidney Wax Bean plot at Earl, and the No. 2 Sensation Wax Bean plot at Barron, Wisconsin. Since these trials were not replicated, results are not of too much significance except to compare with the results from replicated plots. There was an increased yield of 15 per cent from the treated plants over untreated at Earl, and a 26 per cent increase at Barron.

As in 1942, grade records were kept of the 1943⁵ yields from the specific treatments and checks. The average percentage of Grade A wax beans from the Earl dust plot was 68, 71, 67, and 70 for the NA 40, 80, and 160 p.p.m. treatments and the check, respectively; the Rice Lake Grade A percentages

TABLE 10.—*Yields of Wax beans from demonstrational plots in 1943*

Location near	Treatment	Yields from pickings				Total yields	Yield increase
		1	2	3	4		
		Oz.	Oz.	Oz.	Oz.	Oz.	Per cent
Earl	NA dust 80 p.p.m.	272	472	240		984	15
	None	224	416	216		854	
Barron	NA dust 80 p.p.m.	336	736	512	112	1696	26
	None	288	640	336	80	1344	...

were 84, 81, 83, and 85 in the respective cases. The slight differences in percentage of Grade A beans are not significant, thus one can only conclude that the sieve size of beans from the various γ -naphthalene acetic acid-treated blocks was about the same as from check blocks, and that increases in yields of from 8 to 24 per cent must be due to production of more beans rather than larger beans.

DISCUSSION

The falling of buds, blossoms, and small pods has been a serious problem for some time, not only with beans, but with various other plants. The conspicuous success in the prevention of fall of apples (5) is well known and has pointed the way for continuing studies with other crops.

So far as the writers are aware, this paper presents the first evidence that certain kinds of damage from insects can be counteracted by hormones.

Among the difficulties with these lines of work have been (1) the limited season during which blossom drop or related phenomena could be studied out-of-doors and (2) the variability in seasons, which has handicapped quantitative duplication of results. Both have inhibited rapid progress in this important field.

To overcome these difficulties, we have induced the formation of abscission layers rapidly and at will by treatments with illuminating gas. This

technique was used for the first time, as far as we are aware, in our screening tests with various chemicals. It seems to have applications considerably broader than those employed here. However, we do not imply that the formation of abscission layers under the influence (1) of high temperatures and long days, (2) of attacks by certain insects, and (3) of illuminating gas, not to mention natural maturity, are all necessarily identical processes. Nevertheless, the prevention of abscission layers, formed in association with these several influences by α -naphthalene acetic acid, suggests that these processes are at least closely related.

Many comparable problems, such as failure of pod set with lima beans and alfalfa, might be approached with technique similar to that used here. With experiments to repel or to kill certain injurious insects, one might include also materials that would counteract their damaging effect. It might even be pertinent to inquire whether the beneficial effects with apples came entirely from the prevention of abscission layers associated with natural maturity or whether insects might also be helping to hasten the abscission.

The reason for the effectiveness of dusts and the poor results with sprays is rather obscure. Part of the trouble with the sprays came from the excessive pressure employed, which mechanically knocked off some blossoms. In addition, it appears that the hormone came into more intimate contact with the plant, all at once, and then the effect was over soon. Possibly the dust has the advantage of a less vigorous reaction at the time of application and of a longer duration of the effective treatment. Much more hormone can be used in dusts than in sprays. This is fortunate since dusting equipment is more practical for treating this crop.

The advantage is apparent of having a high percentage of the blossoms set on a single stem. For example, a large number of beans will compete against one another and will probably all be in the smaller, high quality, Grade A class. If a smaller number of beans set, they will grow more rapidly between pickings and may easily reach a larger, less desirable, Grade B size.

The strength of application is obviously critical. When the α -naphthalene acetic acid was used at 140 p.p.m., followed by a rain, it was valuable, but the next year when no rain followed, a slightly greater amount (160 p.p.m.) was detrimental. Probably 40 to 80 p.p.m. is better for outdoor application. Much less is effective in a greenhouse where there is little wind.

When too much chemical is applied, various types of injury may be secured, including reduction in yield.

The similarity in activity between the α -naphthalene acetic acid and many other substances, *e.g.*, 2,4-dichlorophenoxy acetic acid, suggests that various other chemicals and other methods of applications are worth investigating.

While we have secured good results with some of these hormone dusts, we recognize the presence of several variables in any commercial bean fields. For this reason the reader will wisely make trials of his own.

SUMMARY

Bud, blossom, and small pod drop of canning beans has reduced the yield in Wisconsin, during hot weather and following attacks by various insects, especially *Lygus oblineatus*. As control measures, various hormones were employed to prevent the formation of abscission layers and subsequent loss.

In laboratory screening tests the formation of abscission layers was induced easily and at will by exposure of the bean plants to illuminating gas, 1 part in 135 parts of air, for about a day at 84° to 86° F. Among the hormones tried, α -naphthalene acetic acid and 2,4-dichlorophenoxy acetic acid were the most promising against abscission layer formation.

When used too strong, these hormones not only prevented the formation of abscission layers, but also induced vein clearing, distortion, and dwarfing of leaflets, which might be confused with virus symptoms. These symptoms commonly appeared in trifoliate developing after treatments, the number being influenced by the concentration.

Lygus oblineatus induced bud, blossom, and small bean drop, and distorted and dwarfed the small leaflets of caged plants in the greenhouse. Some of this loss was prevented by dusting with α -naphthalene acetic acid. Similar results were secured in the field.

About 18 per cent increase in yields of field-grown wax beans was accomplished in 1942 with 2 applications of dust containing α -naphthalene acetic acid at 70 p.p.m. in one case and at 140 p.p.m. in another. Rain followed each application. Dust on Refugee beans and spray on both Refugee and wax beans were not helpful. Sprays were usually detrimental.

More extensive trials in 1943 showed 24 and 12 per cent yield increase, respectively, of wax beans with 2 applications of dusts containing α -naphthalene acetic acid at 40 and 80 p.p.m. but they showed a decrease with dusts at 160 p.p.m. and with sprays. No rain followed these trials. The increase in yield was due to a greater number of small high grade beans rather than to larger beans.

Similar results have been secured in demonstrational plots.

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LESPEDeza ANTHRACNOSE¹

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INTRODUCTION

In the autumn of 1941 considerable leaf spotting and defoliation of some volunteer lespedeza plants were observed at Experiment, Georgia. A later survey showed that this leaf spot was widespread in lespedeza fields in Georgia as well as in parts of North and South Carolina. Later the same disease was reported to the writer by Drs. H. W. Johnson and C. L. Lefebvre as having been found in Virginia. Although the disease is rather widespread and seedlings may be stunted or killed during the spring and early summer months and sometimes many leaflets may be lost, stands are never seriously depleted. So far as the writer is aware, this disease has not been reported or described heretofore.

SYMPTOMATOLOGY

The disease is most conspicuous on the leaflets, on which there may be few to many lesions. In nature the number of lesions usually is small; often there are not more than one or a few on a leaflet. The spots vary in diameter from less than one mm. to one-half cm. and often are half the width of the infected leaflet. The lesions usually are somewhat circular or elliptical but may be angular when delimited by veins or at the tips or margins of the leaflets (Fig. 1, A). Any part of the leaflet may be infected, lesions often being found at the apex, along the margins, or on the midrib or other veins. The midrib or smaller veins may be attacked without the surrounding tissue being involved (Fig. 1, B) in which case the lesions are linear with the longer axis parallel to the vein. On the petioles and stems the lesions vary from circular to linear and usually remain small except on the seedlings where the entire diameter of the stem may be involved for some distance (Fig. 1, C, 2, and 3). The lesions, which are apparent on both sides of the leaflet, vary somewhat in color but commonly approach bone brown above and dark olive below (2). At first the surrounding tissue is the normal green, but very soon it becomes pale lemon yellow to apricot yellow and dries to some shade of light brown, often approaching olive-ocher or honey yellow. The margin (Fig. 1, A) may involve half or more of the width of the leaflet; in fact, it may actually involve more of the tissue than the dark area. One lesion often is sufficient to cause the leaflet to drop; and, when many leaflets are affected, considerable defoliation may result. Brownish setae, approaching black under the hand lens, frequently are visible on one or both sides of the lesion. The lesions most commonly originate between the veins but frequently

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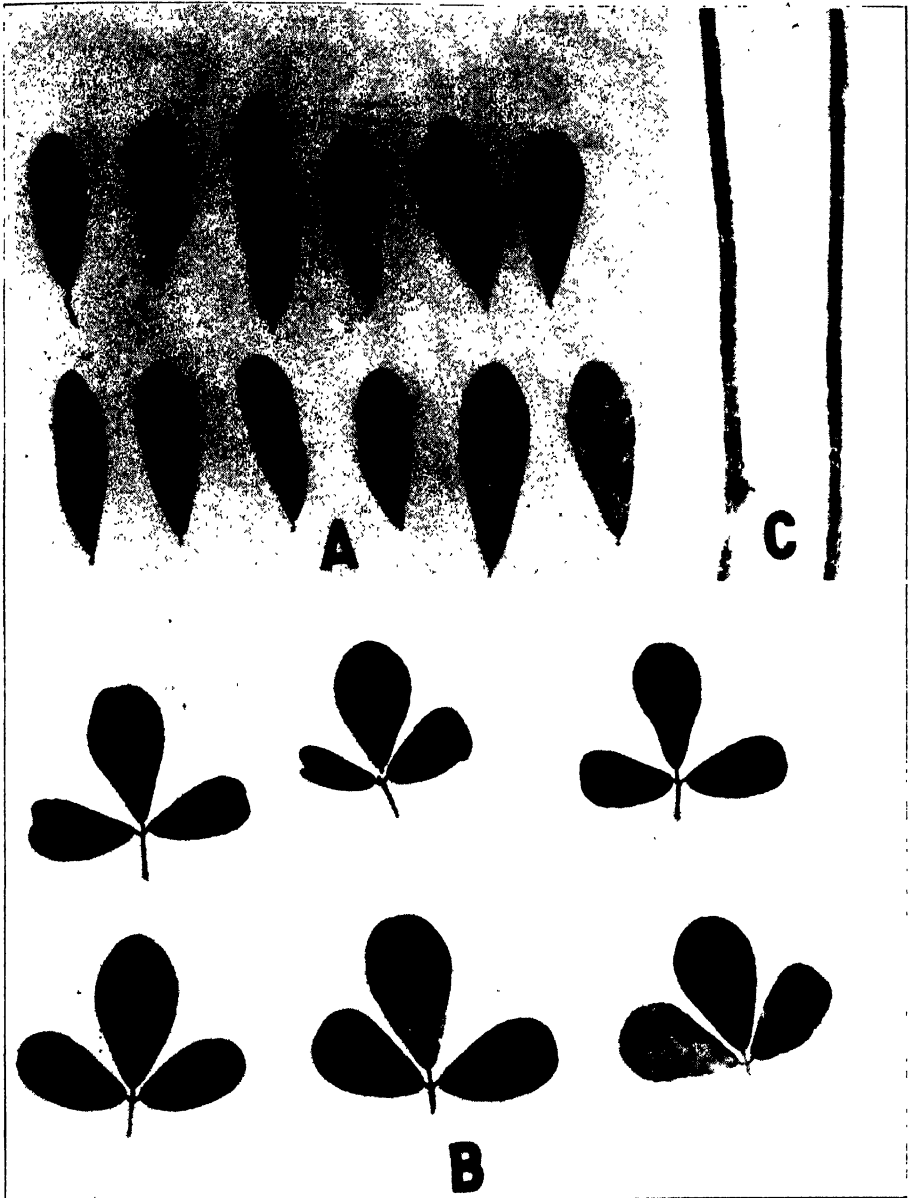


FIG. 1. A. Volunteer annual lespedeza leaflets bearing anthracnose lesions resulting from natural infection. The upper and lower rows of leaflets illustrate the appearance of the lesions on the upper and lower sides of the leaflets, respectively. The midrib may or may not limit the spot. The leaflets that appear light (upper and lower extreme right) were yellowish. $\times 2$. B. Kobe lespedeza leaves with anthracnose lesions resulting from inoculation and 2 healthy control leaves at the left. The upper and lower sides of the leaflets are shown in the upper and lower rows of leaflets, respectively. Photographed 6 days after inoculation. $\times 1\frac{1}{2}$. C. Stems of Kobe lespedeza with numerous black anthracnose lesions, resulting from inoculation, somewhat obscured by the very hairy surface. $\times 2.7$.

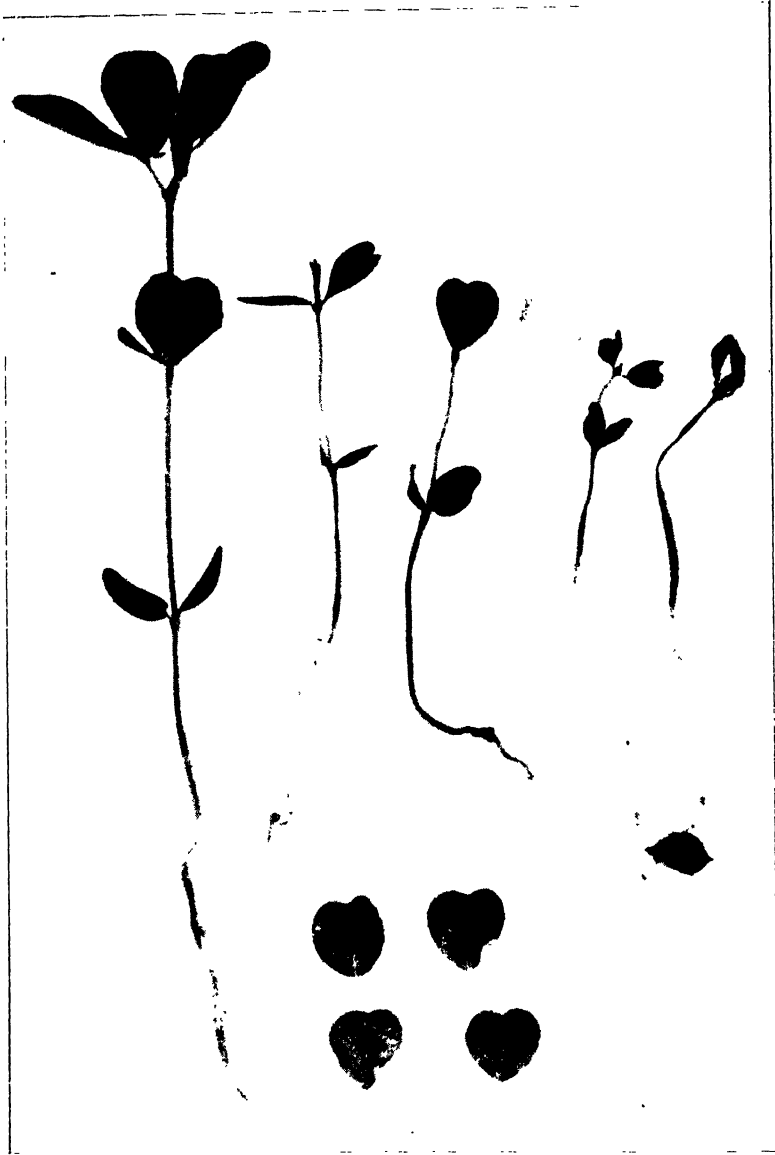


FIG. 2. Kobe lespedeza seedlings. Healthy control at the left, 4 inoculated plants at the right, and 4 inoculated leaflets at the lower center. Photographed 6 days after inoculation. $\times 1.6$.

involve an adjacent vein which then may be slightly more reddish than the remainder of the spot.

ETIOLOGY

Isolations and Inoculations

From lesions on leaflets collected at several places a fungus with the characteristics of a *Colletotrichum* was commonly isolated. The fungus was

studied in pure culture, and inoculations were made to prove its pathogenicity.

The initial isolate formed perithecia sparingly in culture and at first failed to produce conidia. Only a few spores were available, and the first inoculation tests gave negative results. Since the ascospores in culture



FIG. 3. Naturally infected lespedeza seedlings from an experimental plot. Photographed May 3, 1944. The crowns, some of the larger fibrous roots, the stems, and some leaflets had the characteristic anthracnose lesions. The seedling at the left was about normal size although it had a few small lesions. Note the blackened bases of the stems and the lesions higher up on the stems. The plant at the extreme right has a lesion just below the cotyledonary node and 2 lower down. The next plant at the left has a lesion just above the cotyledons. $\times 1\frac{1}{2}$.

resembled those of *Glomerella cingulata*, it was thought that if the fungus would decay apple it might be stimulated to a higher state of sporulation. In order to test this supposition two Winesap apples were inoculated. The apples were disinfected for 5 minutes in mercuric chloride (1-1000) and then were washed in sterile tap water and placed in a sterilized moist cham-

ber. Mycelium from a pure line culture was inserted into a wound made in each apple with sterile tweezers. Two other apples were treated in the same manner except that they were inoculated with a culture of *Glomerella cingulata* from apple, and two others serving as controls were disinfected and wounded but not inoculated. The apples were held at room temperature (15° to 24° C.), and the decay progressed slowly. Final observations made after 6 weeks showed that the apples inoculated with the fungus from lespedeza were entirely decayed, whereas those inoculated with the fungus from apple were only about two-thirds decayed. The controls remained healthy. The fungus from lespedeza was recovered from the apple and used for further studies. On the apple it produced acervuli in which conidia were formed in great abundance. Likewise the reisolate sporulated fairly well on corn-meal agar and especially well on sterilized, green-bean pods.

On June 1, 1943, spores from corn-meal agar were atomized onto lespedeza plants about 2 inches tall which then were held in a moist chamber for 48 hours along with comparable noninoculated plants as controls. No infection was apparent for about 72 hours at which time numerous minute brown spots appeared on leaflets and stems. None of the controls was infected. Six days after the inoculations were made leaflets were yellowing and falling, stems had been girdled by typical brownish cankers, and that portion of the plant above the girdle was dying (Fig. 2). As in nature, one spot proved to be sufficient to cause a leaflet to fall. Although stems become more resistant with age, a single lesion on a very small stem may girdle it and dwarf or kill a young plant.

At the time this work was in progress, 2 other isolates of *Glomerella cingulata* were being studied, one from *Chamaecrista* (6) and one from lupine (5). On July 13, 1943, these 2 isolates, together with the fungus from lespedeza and a culture of *Glomerella cingulata* from apple, were used to inoculate Kobe lespedeza plants about $1\frac{1}{2}$ inches tall and growing in 6-inch pots. The plants were atomized with a heavy suspension of spores from culture, held in a moist chamber at 28° to 33° C. for 24 hours, and then placed outdoors under a cloth shelter. The first evidence of infection was seen after 48 hours on plants inoculated with the fungus from lespedeza. Many more spots were evident the following day, and after 4 days the leaflets were thickly spotted. There was no infection on any of the plants inoculated with the other fungi or on the controls. By the sixth day some of the heavily infected leaflets were turning yellow (Fig. 1, B) and falling and many stem lesions were present. The fungus was reisolated from some of the leaflets and the pathogenicity of the reisolate proved. This experiment not only proves the pathogenicity of the fungus from lespedeza but shows that the pathogen differs parasitically from the isolates from *Chamaecrista*, lupine, and apple.

In another experiment seedlings of several lots of lespedeza were inoculated to test their comparative resistance to this organism. The plants used were growing in 6-inch pots in the greenhouse and were about 8 inches tall.

On October 7, 1943, 11 different lots of lespedeza were atomized with a heavy spore suspension in sterilized tap water, held in a moist chamber for 48 hours, and then transferred to a cloth shelter outdoors. Plants in some of the lots were atomized with sterile tap water and held as controls. Infection was obtained on the following lots of *Lespedeza striata*: (Common) F.C. 22730² and F.C. 31057, (Tennessee 76) F.C. 22664, and (Kobe) F.C. 22731 and F.C. 30935. No infection was obtained on any of the strains of *L. stipulacea* (Korean); namely, F.C. 19601, F.C. 31480, F.C. 65280, F.C. 30888, and F.C. 19604; and none was obtained on the controls. This experiment suggests that the strains of *L. striata* are susceptible and that those of *L. stipulacea* are highly resistant. Field observations have not confirmed this entirely as is shown by the following tentative classification based on ratings made on experimental plantings in the field:

Highly resistant—(Korean) F.C. 31249 and selections 3 and 5 of F.C. 31249.

Resistant—(Korean) F.C. 19601, F.C. 19604, F.C. 30888, F.C. 31481, and F.C. 31485.

Susceptible—(Korean) F.C. 31480 and F.C. 31757.

Very susceptible—(Kobe) F.C. 22731, F.C. 30935, F.C. 22896; (Commercial Kobe); (Common) F.C. 31057 and F.C. 22730; and (Tennessee 76) F.C. 22664.

Just why some of these strains that proved to be susceptible under field conditions did not become infected under experimental conditions is not known. The field data indicate that, although they vary in their resistance, the strains of Korean are more resistant than those of Common and Kobe all of which were rated very susceptible.

Life History

An attempt was made to secure some data on how the fungus can be carried and how new infections are initiated in the spring. Four hundred seeds were divided into 8 lots of 50 seeds each. Four of the lots were planted in pots in steamed soil and without treatment, as controls; 2 lots were dipped into a heavy spore suspension and then planted in steamed soil; and the 2 remaining lots were planted in some of the same soil over the surface of which a heavy spore suspension was poured. The pots were all held in the greenhouse. The seeds were sown Feb. 3, 1944, and the seedlings were removed and examined March 7, 1944. Characteristic lesions were on the underground parts of the stems of some of the plants in the pots inoculated by pouring the spore suspension over the soil, and the fungus was recovered from some of these. A few similar lesions were on the plants grown from the inoculated seed. A very few were on the control plants. In neither of the latter groups, however, was the fungus recovered from any of the lesions; hence, final proof of their exact nature is lacking. There was no doubt that the disease was reproduced by soil inoculation.

² The seed of all F.C. lots were supplied by Mr. Roland McKee, Division of Forage Crops and Diseases, Plant Industry Station, Beltsville, Maryland.

Confirmation of the fact that the fungus can live in the soil and attack the underground parts of seedlings in the field is easily found. On May 6, 1943, dead and dying volunteer lespedeza seedlings 1 to 1½ inches tall were found with typical anthracnose lesions on many of the leaflets and on the stems as well. Some of the stem lesions were sufficiently large and deep to cause girdling; and plants thus girdled were dead, dying, or more or less stunted. Stems with smaller lesions appeared to be suffering little or no injury. Plants were taken to the laboratory, and isolations confirmed the nature of the disease.

On May 11, 1944, a heavy infection by the anthracnose fungus was found in lespedeza plants growing in some of the experimental plots. Typical leaf and stem lesions were present on many of the plants. Some were stunted and a few were dying as a result of the stem lesions. A section of sod about 6 inches square was taken up. The plants were then removed from the soil, washed, and examined. The seedlings were sorted into 3 groups, namely, healthy, slightly diseased, and severely diseased. The plants in the last group were more or less stunted and the stems had fairly deep lesions, especially below ground. The slightly diseased group had shallow lesions and were slightly if at all stunted. This random sample had 28 healthy, 85 slightly diseased, and 130 severely diseased plants, making a total of 88.5 per cent diseased. The healthy plants averaged about 4 inches tall, whereas the severely diseased ones ranged from 2½ to 3 inches. In order to get an idea of what might be the ultimate fate of such plants, 25 healthy, 50 slightly diseased, and 100 severely diseased plants were transferred to pots and held under conditions suitable for good growth. Final observations on June 3, 1944, showed that growth of the healthy plants had been excellent. Some of the slightly diseased ones were somewhat less vigorous, but others were as large as those in the healthy lot. A few of the plants in the severely diseased group died, but many remained stunted, some being ⅓ as tall as the healthy ones. It is evident that, under suitable conditions for the rapid development of the fungus, a high percentage of stem and leaf infection can be expected from the fungus that has lived over winter in the soil or on dead stems. No perfect stage was found on the old stems, and it seems reasonable to assume that the initial infection on the young seedlings in the spring results from mycelium growing in the soil or on the old plant material or from conidia produced on this mycelium. Of course, since the perfect stage developed in culture, its presence in the field cannot be discounted altogether, even though it was not found. Secondary infection probably is caused by conidia from the primary lesions. During dry periods there is little or no fruiting on the lesions, and the disease becomes very inconspicuous or may disappear entirely. It is most abundant during wet periods; and the damage done varies with the amount of inoculum present, the length of the wet period, and the stage of growth during that time. Only very young seedlings have suffered severely from the stem lesions or have been entirely girdled by them.

Effect of Temperature on Mycelial Growth

The effect of temperature on the growth of the fungus was studied in culture on 15 ml. of 2 per cent potato agar plus 1 per cent dextrose in Petri dishes. A loop of a heavy suspension of spores in sterile tap water was placed in the center of each dish, and 5 dishes were held at each temperature. The longest and shortest diameters of the mycelial colonies were measured and averaged at the end of 5 days and again after 10 days. At the lowest temperature (3.5° C.), the spores germinated, but no measurable mycelial colony was formed in 10 days. A small colony was formed at 12.5° C., the fungus grew rapidly at 22° and at 29° C., slowly at 31.5° C., and very slowly at 34.5° C. The spores did not germinate at 38° C. Later observations showed that some growth took place at 12.5° C. and that a small mycelial colony had been formed at 3.5° C. in 20 days. The minimum temperature for growth, therefore, was not reached. The maximum lies somewhere between 34.5° and 38° C. The optimum was somewhere between 22° and 29° C. This experiment was repeated in an effort to determine the optimum more precisely by changing some of the temperatures in that range. Average temperatures of 25.8° and 28° C. were secured. The results of this experiment, as well as the first, showed that the optimum appeared to shift, being higher at the end of 5 days than after 10 days. This is thought to have been due, at least in part, to the drying out of the medium at the higher temperatures. It was decided that the amount of growth during the first 5 days was probably a truer measure of the optimum, because appreciable drying of the medium had not yet taken place. In the second experiment growth was better at 28° than at 25.5° C.; and the optimum lay somewhere between these 2 temperatures, probably a little nearer the former figure, or possibly near 27° C. Shear and Wood (3) in discussing the temperature relations of *Glomerella cingulata* state that "the minimum, optimum, and maximum for the American material was found to be . . . 10°, 27°, and 32° C., respectively." It is probable that the optimum reported by these workers is very near that found by the writer for the fungus from lespedeza. The latter fungus, however, grew at 3.5° and at 34.5° C.; hence, these points differ somewhat from those given by Shear and Wood. Von Schrenk and Spaulding (4) state that the fungus causing the bitter rot of apple develops best at temperatures ranging from 33° to 38° C. This suggests that the upper limit for the growth of this fungus may vary considerably. These writers state also that the minimum for growth of their fungus is 2° C. which is very near the minimum for the lespedeza fungus.

Taxonomy

The original isolate from lespedeza at first formed only ascospores that resembled those of *Glomerella cingulata* (Ston.) Spauld. and Schrenk. These spores were slightly curved and measured $4.2\text{--}6.0 \times 13.6\text{--}23\ \mu$ (av. $4.9 \times 19.5\ \mu$), which is well within the range given by Shear and Wood (3). Conidium-

forming strains were later obtained, and the original isolate was induced to form conidia on decaying apple. These conidia measured $3-6 \times 13-22 \mu$ (av. $4.1 \times 18.2 \mu$), which also is within the range given by Shear and Wood. Setae were present among the spores but varied from few to abundant. They were dark brown to nearly black at the base, often becoming nearly colorless at the apex. The base of the seta is bulbous and is $6-10 \mu$ wide at the broadest part. The seta tapers to a somewhat blunt point, is 70 to 180μ long, and is usually 2- to 4-septate. The fungus differs in this last respect from *Colletotrichum destructivum* O'Gara which is described (1) as having setae which are often nodulose and continuous or obscurely 1-septate. Since no morphological difference was found that would enable one to distinguish the fungus on lespedeza from *Glomerella cingulata*, it is considered to be a strain of this species. The fact that no infection of lespedeza was obtained with the isolate of *G. cingulata* from apple, while abundant infection was obtained with an isolate from lespedeza under identical conditions, indicates that the latter may be a different parasitic strain.

CONTROL

The damage caused by the anthracnose fungus to lespedeza in the field is rather limited. What information is available indicates that serious damage to lespedeza is caused only under rather wet conditions, especially in the spring when the seedlings are small. Should the disease become sufficiently important to justify control measures, it seems probable from the results of inoculation tests and field observations that highly resistant varieties can be found, especially among the Korean strains such as F.C. 31249.

SUMMARY

An anthracnose of annual lespedeza caused by a strain of *Glomerella cingulata* is described. The disease causes more or less circular, elliptical, or angular brownish lesions from 1 to 5 mm. in diameter on the leaflets. The number of lesions per leaflet may vary greatly, but a single leaf spot is sufficient to cause a leaflet to drop off. The lesions on the petioles and stems are dark brown to nearly black and vary from circular to linear. They are of importance largely on young seedlings, whose stems may be completely girdled and the plants killed. The disease may be very prevalent in local areas during wet periods, especially during the late spring and early summer months, and may disappear during subsequent dry spells. The strains of *Lespedeza striata* appear to be more susceptible than those of *L. stipulacea*, although some of the latter are moderately susceptible. It is thought that, should control measures become necessary, resistant varieties may be found among some of the strains of the latter species such as F.C. 31249.

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RESPONSE OF SPRING BARLEY VARIETIES TO FLORAL LOOSE SMUT INOCULATION^{1, 2}

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Loose smut of barley caused by *Ustilago nuda* (Jens.) K. and S. occurs generally wherever barley is grown, but is more prevalent in the humid and semi-humid areas of production. Losses are usually small in terms of percentage reduction of crop yield, but may be heavy at times. Several barley varieties widely grown in the North Central area of the United States are susceptible to *U. nuda*. With the exception of Trebi, no resistant variety is widely grown in this area. The organism causing loose smut can be controlled by hot-water treatment of the seed, but this method is both difficult and hazardous in application. It would be impractical for growers to treat with hot water all barley used for seed. An easier way to control the loose-smut pathogen would be the use of adapted resistant varieties, if such were available.

For several years the loose-smut disease has been given consideration in the barley-breeding program at the Wisconsin Agricultural Experiment Station. Attention has been given toward finding varieties with good agronomic type as well as resistance to loose smut and other important diseases. It would be desirable for new varieties to carry a practical type of resistance so as to prevent natural loose-smut epidemics.

LITERATURE REVIEW

Various techniques have been used for artificial inoculation with loose smut since Maddox (7) first showed that he could produce loose smut of barley by putting the spores on the ovary of the plant at flowering time.

Brefeld and Falek (1) used three methods to inoculate with the loose smut of wheat and barley. An atomizer was used to blow smut spores around the heads which were enclosed in a glass cylinder, producing from 9.5 to 20 per cent smut. Other methods included the introduction of either dry spores or a spore suspension into flowers by use of a small brush. Infection varied from 13 to more than 99 per cent smutted plants.

Freeman and Johnson (4) clipped the lemma and palea near the base of the awn, leaving an opening for introducing spores. In some cases dry spores were placed directly on the stigma with a brush or forceps, and this was their most effective method. Some heads were covered and others were moistened after inoculation. Optimum infection period was from full bloom until the ovary began to enlarge.

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³ The writers acknowledge valuable assistance by D. C. Army in the field work and assembling the data; and by V. I. Hack in the field inoculations. J. G. Dickson gave counsel in planning the project, and J. H. Torrie aided in statistical interpretation.

Broili (2) opened the flowers with a forceps or cut off the upper one-third of the lemma and palea and then blew in spores with an atomizer. He also employed Freeman and Johnson's method of opening the flowers with a forceps and dusting on spores with a brush (3). Seiffert (16) used Brefeld and Falek's brush method and a hollow needle resembling a spoon. The needle was dipped into spores, forced between the lemma and palea and the spores blown into the flower. He listed the varietal reaction from 0 to 94.4 per cent smut.

In his work with wheat loose smut, Piekenbrock (12) described an apparatus where dry spores were blown into the flowers by a blast of air produced by moving a piston in a cylinder. Modifying one of Freeman and Johnson's methods, Tapke (19, 20) inoculated barley flowers with loose smut by gently placing, with forceps, a small quantity of spores on the stigmas of flowers in the process of blooming. With high humidity after inoculation, Multan barley (19) developed 87 per cent smutted plants, and none under desert conditions. Zehner and Humphrey (24) obtained 33 per cent infection in Tennessee winter barley by injecting tillers in the apical growth region with a 14-hour spore suspension of *Ustilago nuda*. Zeiner (25) used Piekenbrock's method for testing parental and progeny responses for several barley crosses. Infection was from 0 to 78 per cent, and he also substantiated Piekenbrock's observations with wheat that the most favorable time for inoculation was shortly after flowering until 5-7 days after pollination. Nahmmacher (10) used a modification of Piekenbrock's apparatus, a rubber bulb replacing the piston and cylinder. Varietal reaction, inheritance of resistance, and physiological specialization studies were made where he differentiated 2 races of *U. nuda* on a pathogenicity basis. Vanderwalle (22) used a micropipette to inject a suspension of mycelium into the embryo. Developing plants were smutted, but it is not clear whether the fungus was *U. nuda* or *U. medians* Bied. since he (23) later mentioned sporidium formation. Peck, according to Thren (21), clipped the awns and treated the entire head *in vacuo* with dry spores. Moore (9) introduced a spore suspension into flowers of heads placed in a small glass chamber where pressure could be reduced. Wheat was more successfully inoculated than barley. Thren (21) used Nahmmacher's method for inoculating barley flowers, reporting high infection and increased survival counts when live-spore concentration was reduced. Oort (11) used the partial vacuum method of Moore for inoculating wheat and barley heads, and found the optimum time for infecting barley was near time of pollination. Lange-de la Camp (5) inoculated wheat flowers with a suspension of mycelium of *U. tritici* (Pers.) Rost. and kept heads moist for a few days. This is probably the first report of mycelium inoculation being successful. Middleton and Chapman (8) inoculated winter barley, using the vacuum and forceps methods. Livingston (6) used Moore's method and then maintained inoculated plants 72 hours in a chamber with 85-95 per cent relative humidity and at 15-30° C. Inheritance of resistance was studied. Semenink and Ross (17) enclosed

dry spores in 4 layers of cheesecloth and dusted heads of Newal barley several times. Poehlman (13) described a method using a spore suspension which was injected into individual flowers by a hypodermic needle. Ross and Semeniuk (14, 15) have also used this method. These workers have tested varieties and their results generally agree with those about to be reported in this paper.

METHODS AND MATERIALS

Inoculations

The present work with *Ustilago nuda* was reemphasized in 1936. Several methods were used in order to learn the one which would be most effective and most easily applicable. When inoculating barley heads it must be remembered that the individual flowers of a head are not all in the same stage of development. Several of the previously cited German workers removed small and lateral flowers. In the present work all flowers were left on the head with the exception of the very smallest ones at the base of the spike. One method used was the partial vacuum described by Moore (9). This was modified by using a larger glass tube than previously described in order that 2 or 3 and sometimes 4 heads could be inoculated at the same time. In addition, a solution of malt extract was used in order to increase germination and growth of the fungus after inoculation. A small amount of fish-oil soap was included in the spore suspension so as to decrease surface tension, permitting thorough wetting of the ovary and stigma.

A second method used was that first reported by Freeman and Johnson and later modified by Tapke where forceps carrying smut spores on finely-ground points were used to pierce the lemma and gently touch the stigma. A third method was that of clipping the upper part of the lemma and palea and dusting the inside of the flower with smut spores by means of a camel-hair brush. This type of inoculation had been previously used at Wisconsin in studying stripe of barley caused by *Helminthosporium gramineum* Rabh.

These three methods of inoculation were used in 1936 and 1937⁴ and a portion of the heads were covered with glassine bags. The summary of the results is given in table 1. The bagging seemed to reduce smut infection during the 2-year period, when more than 900 inoculations⁵ were made each year. Bagging was therefore discontinued for the inoculations beginning in 1938.

A fourth method of inoculation was introduced in 1938 when flowers were inoculated by use of a hypodermic needle, to which was attached a small rubber bulb containing dry spores of *Ustilago nuda*. After the needle pierced the lemma the bulb was squeezed in order to puff spores into the ovary. This is hereafter referred to as the "needle" method. In 1940, reference was made to this method in the annual report of the Wisconsin Agricultural Experiment Station.⁶

⁴ The results were read the following year.

⁵ In this paper the inoculation of all flowers of 2 barley spikes generally was considered a single inoculation.

⁶ Annual report of the director of Wisconsin Agricultural Experiment Station. Bull. 449, p. 74.

TABLE 1.—*Influence of methods of inoculation on development of Ustilago nuda when many varieties of barley were used*

Year grown ^a	No. of inoculations ^b	Method of inoculation and percentage of smutted plants								
		Vacuum			Forceps			Clipped ^c		
		Bagged	Not bagged	Ave.	Bagged	Not bagged	Ave.	Bagged	Not bagged	Ave.
1937	150 ^c		16.04			6.76			11.89	
1937	907 ^d	6.5	9.1	8.8	0.4	4.3	4.0	2.3	8.6	7.7
1938	954 ^d	15.0	29.1	25.2	15.1	19.9	17.9	6.6	9.2	8.5

^a Inoculations made the previous year.

^b Two heads generally used for an inoculation.

^c The same 50 varieties inoculated by each method.

^d Large number of varieties not strictly comparable for each method.

^e Upper part of lemma and palea clipped before dusting with spores.

A comparison of the needle and partial-vacuum methods with a large number of varieties is given in table 2. The results reported in tables 1 and 2 are not strictly comparable; but since the number of inoculations is large the results are thought to be fairly reliable. The needle method (Table 2) produced considerably more smut in the 1938 inoculations, the results of which were read in 1939. In 1940 the two methods gave very nearly the same percentage infection. Inasmuch as the needle method is much more easily used than the vacuum method, the latter was discontinued after the 1939 inoculations.

Since the data obtained from many varieties were composited in tables 1 and 2 it was thought desirable to compare the four methods of inoculation on Wisconsin Barbless and Oderbrucker varieties. These results, summarized in table 3, indicate that the needle and vacuum methods were more effective than the others. Only two years, 1938 and 1939, are available for comparison of the needle and vacuum methods. The vacuum method produced lower smut percentages than the needle method and was more difficult to use. Including Odessa, the needle method gave more infection for 5 of the

TABLE 2.—*Comparison of vacuum and needle methods of inoculation for Ustilago nuda, with many varieties of barley*

Year grown ^a	Method, number of inoculations ^b , and smutted plants			
	Vacuum		Needle	
	Inoculations	Smutted plants	Inoculations	Smutted plants
	<i>No.</i>	<i>Per cent</i>	<i>No.</i>	<i>Per cent</i>
1939	635	24.4	181	42.3
1940	550	21.9	299	22.8

^a Inoculations made the previous year.

^b Two heads generally used for each inoculation.

6 varietal-year comparisons, with the exception of the 1940 results with Oderbrucker. While the summarized results should not be compared strictly, a total is given for the number of plants observed for all of the years and the percentage smut.

TABLE 3.—*Comparisons of inoculation methods when three barley varieties were inoculated with Ustilago nuda from day of pollination to 3 days after pollination*

Year grown ^a	Needle		Vacuum		Forceps		Clipped	
	Plants		Plants		Plants		Plants	
	Total	Smutted	Total	Smutted	Total	Smutted	Total	Smutted
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Wisconsin Barbless (C.I. 5105)								
1937	1919	7.2	1247	1.0	1805	4.9
1938	1601	16.4	359	1.4	852	2.3
1939	501	33.1	1163	26.0
1940	203	22.2	684	18.0
1941	1084	44.1
1942	267	15.0
1943	751	41.1
1944	86	58.8
Total or Av. ^b	2892	37.6	5367	15.4	1606	1.1	2657	4.1
Oderbrucker (C.I. 4666)								
1937	314	16.9	130	16.2	120	3.3
1938	115	36.5	357	4.5	157	12.1
1939	514	49.2	1019	32.2
1940	480	14.0	708	34.3
1941	1443	52.3
1942	535	37.0
1943	614	56.7
1944	429	69.9
Total or Av. ^b	4015	47.8	2156	30.9	487	7.6	277	8.3
Odessa (C.I. 934)								
1937	150	28.0
1938	321	36.1
1939	43	32.5	223	5.8
1940	53	58.5	163	43.6
1941	239	23.8
1942	10	90.0
1944	55	92.7
Total or Av. ^b	400	40.5	857	28.2

^a Inoculations made the previous year.

^b Total number of inoculations or average percentage of smut.

The stage of floral development at the time of inoculation is important. The different stages of development, using the two methods, needle and vacuum, are compared for susceptibility in table 4. The results for the needle method are given for a 6-year period from 1939–1944 and for the vacuum method for 3 years, namely 1938–1940. The results indicate that the barley flowers are most susceptible during and shortly after pollination.

Inoculations made later than 3 days after pollination gave a reduced infection. When Wisconsin Barbless was inoculated 4 days after pollination, smut was reduced to 6.5 per cent, and Oderbrucker infection was reduced to 5.1 per cent after 5 days following pollination. It is impractical to inoculate spikes before pollination because the heads have to be removed from the boot and the necks are rather weak and tender at this time. Therefore, it is desirable to wait until slightly after pollination. Nearly all of the inoculations were made near the time of pollination and since 1940 not later than 3 days after pollination.

Floral inoculation often affects the subsequent seed and plant development, frequently reducing germination and seedling vigor. If flowers are too young at time of inoculation, seed set is reduced more than when flowers

TABLE 4.—*Influence of stage of floral development on infection by Ustilago nuda, using two varieties of barley and two methods of inoculation*

Stage of floral development with reference to pollination	Wisconsin Barbless				Oderbrucker				Average infection, both varieties	
	Needle 1939-44		Vacuum 1938-40		Needle 1939-44		Vacuum 1938-40		Needle 1939-44	Vacuum 1938-40
	Plants	Smut	Plants	Smut	Plants	Smut	Plants	Smut	Smut	Smut
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	Per cent	Per cent
Day before	111	45.9			179	60.9			51.1	...
Day of	1334	41.4	217	36.9	1692	51.7	87	50.6	46.6	43.8
1 day after	1093	30.8	702	17.8	1687	47.3	555	35.5	39.1	26.7
2 days after	246	38.2	1405	21.7	321	31.8	656	27.6	35.0	24.7
3 days after	57	57.9	1124	15.8	126	25.4	514	35.6	41.7	25.7
4 days after			306	6.5			70	25.7	...	16.1
5 days after			169	8.9			58	5.1	...	7.0
6 days after			39	7.7						

have been pollinated for a day or more. Such seeds are frequently light and chaffy, and secondary fungi may reduced stands. Treating inoculated seeds with organic mercury dust before planting reduces seedling losses and increases vigor. Hulls are often loose and fail to adhere to the caryopsis. Usually hulls are loosened most with lemma clipping and least with the vacuum technique. Plant stands vary from year to year when the same method is used, many factors probably contributing to the variation. Stands varied from 40 to 72 per cent and averaged 53 per cent with the needle method, from 1939 to 1944 inclusive. In 1939 and 1940 the vacuum inoculations produced 8 per cent greater stands. Thren (21), by reducing live-spore concentration to 1 per cent, raised the stand counts and yet maintained good infection.

The needle method was chosen by the authors because it produced the most infection and could be manipulated by one technician. Needle size found most successful was 20 gauge. Inoculum should be finely sieved and kept

dry, otherwise the needle may clog, which necessitates cleaning by a small wire. Reduced live-spore concentrations would likely increase stand counts and yet maintain high infection. The needle could also be used for injecting a spore suspension, though not done to a great extent in this work.

Inoculum

A composite inoculum was obtained from susceptible varieties grown currently in the nursery. Inoculum was collected from many varieties so as to maintain various races of the fungus which may be present in the nursery. Although specialized races are known in many species of *Ustilago* and also exist in *U. nuda*, no carefully purified race was used, thus avoiding a restriction in the application of the results. In rare cases smut used had been previously stored at low temperatures. Samples of the smut were germinated in order to determine purity of species, viability, and vigor. Floral inoculation with *U. nuda* only is reported in this paper.

Barley Varieties Tested

A large number of the varieties came from the C.I. collection.⁷ The seed was originally supplied by R. G. Shands. Previous to the loose-smut test most of the varieties had been found resistant to the two sporidium-forming smuts. A large portion of these varieties has been inoculated with the stripe-causing fungus and the results reported by Shands and Arny (18). Many selections from the barley-breeding program in progress at the Wisconsin Agricultural Experiment Station have been tested for their reactions to *Ustilago nuda*. Like their parents, most of the hybrid selections were susceptible. The selections having numbers preceded by "H" were provided by R. G. Shands.

The varietal testing for reaction to *Ustilago nuda* has been in progress for about 10 years, and some of the resistant varieties have been hybridized with agronomically desirable varieties and their progenies have been tested for resistance. A few smooth-awned selections with resistance to *U. nuda* have been purified and are being tested for yield and adaptability.

RESULTS

Tests were from field inoculations and nearly all were field-grown. Only a small portion was grown in the greenhouse. Even though fairly extensive tests were made before 1939, the data in tables 5 and 6 give results mostly with inoculations since that time. The percentages of smut in Oderbrucker and Wisconsin Barblless for the period of 1939-1944 are 48 and 38, respectively, while some other varieties had 90 per cent or more, and still others had less than 5 per cent, thus indicating a satisfactory method for differentiating varietal reactions. The partial-vacuum technic was used for testing many hybrid selections and varieties, but the results were omitted except where the variety was not later tested with the needle method. Varieties or

⁷ A world collection of barley varieties maintained by the Division of Cereal Crops and Diseases, U. S. Department of Agriculture.

TABLE 5.—(Continued)

C.I. No. and description ^a	Tests	Plants		C.I. No. and description ^a	Tests	Plants			
		Total	Infected Wt. Ave.			Total	Infected Wt. Ave.		
	No.	No.	Per cent		No.	No.	Per cent		
2135 ^c	2	4	188	42	2877	2	12	58	
2203	H	3	86	7	2947 ^c	21	842	24	
2207	H, N	3	75	33	3208-4	11	329	1	
2208	B, H, N	4	79	0	3210-2	11	271	1	
2209	A	3	83	2	3393-2	2	22	27	
2213	I, N	9	238	32	3402	6	60	3	
2215	1, H, N	2	82	42	3403 ^c	5	244	58	
2219	2, H	3	31	13	3404	4	86	22	
2221	2, N	2	39	54	3408	2	28	14	
2223	2, H, N	4	60	5	3410	2	35	66	
2226	D, B	6	101	0	3413	A	2	28	43
2237	A, II	2	51	42	3443	4	68	44	
2242 ^d	P, II, N	3	55	6	3446	1	19	74	
2247	A, N	2	35	31	3508	4	60	17	
2253	N	6	120	23	3530	1	47	85	
2254	I	2	51	39	3552	5	87	95	
2257 ^d	N	5	92	1	3553	3	59	86	
2261	N	5	55	7	3554	4	110	81	
2263	P	4	94	4	3556	1	10	70	
2267	A	2	41	37	3557	3	33	88	
2276	B	11	347	.9	3558	4	103	89	
2277	P, N	4	70	4	3559	4	35	17	
2278	A	1	56	39	3560	2	23	83	
2280	I	2	24	29	3582	4	80	63	
2282		2	65	51	3634	3	40	88	
2284	A, B	7	135	4	3650	4	105	71	
2286	I, N	7	177	34	3724	2	48	54	
2318	N	6	89	2	3728	4	99	59	
2321	H, N	2	31	42	3745	4	35	31	
2330		1	68	59	3746	2	44	66	
2338	B	4	68	12	3776	3	29	66	
2344		2	23	39	3778	4	42	74	
2348		3	43	35	3808	2	53	100	
2351	N	4	66	?	3810	3	75	76	
2352	I, N	6	127	32	3816	4	39	77	
2353	B	4	146	19	3895-1	2	38	97	
2364		5	160	60	3896-1	1	10	90	
2381	A	5	130	23	3898	1	8	88	
2383	B	4	86	7	3902	2	55	89	
2432		5	127	32	3903	3	36	89	
2433		12	255	17	3905-1	B	3	55	38
2437	2	2	23	83	3906-2	B	3	37	27
2448	N	16	470	3	3906-3	P	2	25	28
2467		11	280	36	3912-3	B	1	18	89
2476		4	114	40	3914-1	P	1	22	23
2480		4	94	49	3921-1		2	47	66
2481		5	77	49	3921-2		1	7	57
2482	H	4	76	63	3924-2		2	40	43
2492		5	374	50	3969-1		1	41	34
2497	B	5	64	0	3971		2	57	74
2525		2	63	13	4032		2	74	80
2526		2	19	63	4033		2	51	100
2542		2	32	19	4033-1		2	67	88
2573	N	2	22	18	4038		3	54	96
2577	N	2	42	41	4041-2		2	75	36

TABLE 5.—(Continued)

C.I. No. and description ^a	Tests	Plants		C.I. No. and description ^a	Tests	Plants		
		Total	Infected Wt. Ave.			Total	Infected Wt. Ave.	
	No.	No.	Per cent		No.	No.	Per cent	
4049	1	17	82	5612	2	33	37	
4118	8	170	74	5641	2	19	42	
4184	2	37	65	5643	2	62	92	
4201-1	2	43	49	5696 ^c	B	3	51	31
4211	2	20	65	5841	A	1	21	19
4211-1	1	10	50	5899	P, N	10	167	6
4223	1	16	88	5998	S	1	20	35
4252	S	9	301	6001 ^c	6	235	26	
4289	11	487	34	6036	S	2	70	34
4376	D, B	2	56	6051	H	4	140	74
4425-1	4	110	30	6087	2, SS	3	44	71
4426	1	23	48	6088	S	12	369	68
4502-2	7	170	14	6093	S	5	213	43
4559	9	221	8	6109	S	4	112	25
4559-1	3	67	2	6199	1	18	100	
4560-1 ^c	4	122	10	6239	S	9	293	41
4561	3	82	12	6251	10	241	40	
4573-1	2	27	7	6299	S	1	17	82
4576-1 ^c	6	207	15	6311	1	15	80	
4577 ^c	S	18	691	6352	1	46	11	
4577-1	S	5	164	6395	2	1	18	72
4585	2, S	6	132	6397	2	1	31	94
4585-1 ^c	5	89	6	6421	2	1	25	28
4617 ^c	6	183	29	6425	2	1	12	92
4622	11	197	16	6430	2	1	18	72
4623	17	341	13	6464	2	1	12	100
4624	2	39	8	6467	1	13	77	
4633	3	33	70	6487	1	13	77	
4637	7	137	42	6488	2	1	11	91
4666	132	4052	48	6503	6	286	48	
4687	II	5	195	6531	2, B, S	8	132	60
4726 ^c	6	265	24	6544	7	264	38	
4731	2	45	18	6572	N	13	272	13
4757	1	39	46	6607	1	33	36	
4801 ^c	2	4	132	6608	1	56	39	
4820	1	44	68	6609	1	42	36	
4821	B	16	386	6611	1	54	37	
4822	P, N	9	194	6614	3	66	2	
4823	B	16	327	6615	1	49	38	
4827 ^c	5	175	38	6991	A	4	115	0
5027	2, S	3	55	7011	S	2	48	83
5030	10	389	48	7015	S	4	60	65
5105	S	106	2892	7030	S	5	155	71
5221	4	78	49	7055	2	48	2	
5267	9	364	60	7069	S	7	298	36
5346	3	143	25	7115	S	4	112	67
5409	2	33	36	7116	S	4	119	24
5419 ^c	D	3	86	7143	S	2	60	65
5459 ^c	D	3	80	7165	3	149	24	
5581	1	23	78	7166	13	570	20	

selections having less than 20 plants for observation were omitted from tables 5 and 6 unless susceptibility was clearly denoted. When a variety was susceptible it was not tested further unless desirable from some breeding standpoint.

TABLE 6.—*Loose smut reactions of barley hybrid selections and miscellaneous varieties when artificially inoculated with Ustilago nuda*

Hybrid selections ^a or varieties and description ^b	Tests	Plants		Hybrid selections ^a or varieties and description ^b	Tests	Plants			
		Total	Infected Wt. Ave.			Total	Infected Wt. Ave.		
	No.	No.	Per cent		No.	No.	Per cent		
X154-2-79		11	476	34	X301-1	S	3	79	44
X173-5-4-1	S	7	227	59	H4-5-1-3-11-4	S	3	114	38
X173-10-5-6-1	S	11	442	51	H4-8-7-3-1-1	S	5	203	67
X182-8-1-2	S	13	447	61	H46-1-4-4-6 ^d		2	43	23
X182-8-2-8	S	6	205	72	H47-91		2	48	6
X182-8-3-3	S	5	162	62	H80-4-21		2	65	79
X182-8-3-4	S	6	170	69	CC 12	S	7	93	0
X182-8-3-7	S	6	163	59	CC 29		2	53	64
X182-79-1-1	S	3	122	57	CC 146		6	176	19
X182-243-1	S	3	78	33	WxWx		2	94	66
X184-5	S	4	127	64	Wxwx		3	101	68
X184-11-15-1	S	4	108	45	wxwx		6	203	58
X191-3-3	S	1	36	31	Trebi Sel. (many leaves)	6	115	0	
X191-16-2-1	S	9	286	68	S.S. 1314		1	73	34
X191-16-3-1	S	9	347	71	Lion Wis. 117-28	R, S	5	147	38
X191-24	S	6	242	57	Michigan 110	2, S	7	123	33
X191-25	S	7	229	49	Olli x Asplund		6	210	44
X207J-3-1	S	3	61	7	Tammisto 03639		2	35	31
X207J-3-3	S	3	107	8	Tammisto 04432		5	117	47
X212-1	S	4	152	30	Tammisto 09234		4	141	58
X217-27	S	3	97	16	Belgian	2	4	43	67
X217-57-1	S	3	61	2	CP 170	2	6	173	45
X217-62	S	3	35	0	CP 127422	2	10	265	2
X226-2	S	2	31	66	Georgine Ped.	2	7	181	55
X265-1	S	1	30	17					

^a Parentage of hybrid selections are as follows:

- X39 —Oderbrucker (Wis. Ped. 5-1) x Lion (Wis. 117)
- X154 —Oderbrucker (Wis. Ped. 5-1, C.I. 4666) x Korsbyg (Wis. 97-3)
- X168 —X39-5-8-4-1 x Oderbrucker (Wis. Ped. 6, C.I. 1529)
- X173 —X39-9-3-6-8 x Oderbrucker (Wis. Ped. 5-1)
- X182 —Wis. Barbless (C.I. 5105) x Olli (C.I. 6251)
- X184 —Wis. Barbless (C.I. 5105) x Pentland (C.I. 5267)
- X191 —Wis. Barbless (C.I. 5105) x Newal (C.I. 6088)
- X207J —Trebi (C.I. 936) x Wis. Barbless
- X212 —Chevron (C.I. 1111) x X168-5-1
- X217 —Trebi x Wis. Barbless
- X226 —H4-8-15 x X182-21-2
- X265 —Wis. Barbless x Pillsbury (C.I. 7166)
- X301 —X182-8-2-8 x P. (Trebi x Pillsbury, C.I. 7166)
- H4 —Wis. Barbless x Chevron
- H46 —Chevron (C.I. 1111-5) x Brachytic (Minn. 78-4, C.I. 6572)
- H47 —Chevron x Trebi (C.I. 936)
- H80 —Chevron x Hillsa (C.I. 1604)

^b See table 5 for description.

The results of varietal tests are given in table 5 for C.I. numbered varieties, and in table 6 for hybrid selections and miscellaneous varieties. The C.I. number, description, the number of tests, the total number of plants observed, and the average percentage of infected plants on a weighted basis are given. If 2 or more tests are recorded they probably were from different years. The weighted average was determined by use of the total plants

in all tests of an individual variety. Names and descriptions of a large part of the tested C.I. varieties were given by Shands and Army (18). Nearly all of the selections from hybrids listed in table 6 are 6-rowed and smooth-awned. A small portion of the miscellaneous varieties listed in the latter part of table 6 are 2-rowed, and nearly all are rough-awned.

DISCUSSION

The two varieties of barley used most frequently as inoculated controls in these experiments are Oderbrucker and Wisconsin Barbless. Oderbrucker had 48 per cent infection and Wisconsin Barbless had 38 per cent. This represents 132 and 106 tests, respectively, of about 30 plants per test. Infection percentages varied within wide ranges, but it is thought that these averages are fairly representative for these varieties.

In order to obtain an estimate of the variability present and the percentage of smut necessary to separate varieties, the analysis of variance was applied to the results of 40 varieties that had been tested for 4 consecutive years. The varieties were placed into two groups, those with less than 20 per cent or more than 80 per cent infection and those which had more than 20 per cent and less than 80 per cent infection. For the first group the smut percentage necessary to separate varieties was lower than for the second group, decreasing as infection approached 0 or 100 per cent. Variability within a variety as measured by the standard deviation was 7.7 per cent for the first group and 14.9 for the second. On the basis of the 4 years, the amount of infection necessary to differentiate varieties in the first group was 11 per cent, and 21 per cent for the second. While these values cannot be applied directly to the results in tables 5 and 6, they can be used as a guide in interpreting the data and for the separation of the varieties. The values would be larger for comparisons including less than 4 years' data and smaller for comparisons including more than 4 years' data.

In testing 300 varieties from the C.I. collection a total of 4 had 100 per cent smut. These four varieties were C.I. numbers 3808, 4033, 6199, and 6424. Of more interest, however, from a plant-breeding standpoint are 36 varieties which had less than 5 per cent infection. In this group 9 varieties had no infection; they are as follows: C.I. 668, 967, 1032, 1223, 1443, 2208, 2226, 2497, and 6991. Evidently these were resistant, but infections might occur under conditions of more extensive inoculations or with different races. The 300 varieties were rather evenly distributed in different infection classes from 0-100 except for the 30-40 per cent groups and the classes containing less than 5 per cent.

It is of interest to note the place of origin of the varieties in relation to their smut reactions which are given in table 7. Abyssinia was the place of origin of the largest number of resistant varieties, while varieties from Egypt and North Africa were generally susceptible. No tested variety from Russia was highly resistant. Several of the varieties from Manchuria had moderate resistance. China and India had a few varieties fairly resistant and several fairly susceptible.

TABLE 7.—*Source and number of varieties in various infection classes when artificially inoculated with Ustilago nuda*

Source or country	Number of varieties in 5 infection classes				
	0-3	4-15	16-30	31-60	61-100
Abyssinia	8	7	6	5	3
Asia		3	1	3	1
Australia	1	2	1	1	3
China	3	1	1	9	..
Egypt			1	3	18
India	2	6	6	7	..
Japan		2	2		..
Manchuria	1	8	7	4	1
Middle East		2	2	3	1
North Africa		2	2	6	13
Northern Europe		3	1	14	9
Russia			4	11	8
Southern Europe		1	1		2
Total	15	37	25	66	59

Most of the hybrid selections given in table 6 were tested for yield as well as for several diseases. Some of these hybrids were made before the parental disease reactions were learned, and some were made primarily for quality factors. Therefore, many of the selections have no appreciable resistance to *Ustilago nuda*, while those with resistance have derived it from either Trebi (C.I. 936) or Dorsett (C.I. 4821).

In table 8 is given the loose-smut reaction of certain commercial barley varieties grown in the United States and Canada. Most of the commonly-grown varieties were relatively susceptible. Trebi and Tregal were resistant, and Warrior had no infected plants, although the latter would doubtless have some infection if studied more extensively. The Manchurian varieties varied in their responses to loose smut, O.A.C. 21 having 15 per cent and Minnesota 184 having 59 per cent infected plants. Peatland, Mars, Newal, Plush, and Missouri Early Beardless are considered as susceptible

TABLE 8.—*Loose-smut reactions of some commercial barley varieties grown in the United States and Canada*

Variety	C.I. No.	Smut	Variety	C.I. No.	Smut
Per cent			Per cent		
Bay*	7113	32	Olli	6251	40
Glabron	4577	23	Peatland	5267	60
Hannchen	531	44	Plush	7030	71
Kindred*	6969	78	Plter	6036	34
Manchuria N.D. 2121	2947	24	Regal	5030	48
Manchuria Minn. 184	2330	59	Spartan	5027	49
Manchuria O.A.C. 21	1470	15	Trebi	936	4
Mars	7015	64	Tregal*	6359	0
Missouri Early Beardless	6051	74	Velvet	4252	26
Newal	6088	68	Velvon	6109	25
Oderbrucker	4666	48	Warrior	6991	0
Odessa	934	41	Wisconsin Barbless	5105	38

* Data by R. G. Shands.

varieties. Other varieties had less infection but are considered moderately susceptible.

In breeding new varieties of barley, it is desirable for the new selections to be resistant to more than one of the major diseases. Shands and Arny (18) gave the stripe reactions for a fairly large number of barley varieties. The loose-smut reaction is given herein for 139 varieties recorded in that paper. There were 17 varieties that had less than 15 per cent stripe and loose smut, a reaction that was considered resistant to each of the diseases. The following are the varieties and their C.I. numbers:

C.I. 936 Trebi	C.I. 2318 Kharsila	C.I. 6352 Cebada 97A
1969 Chinese Black	2338 Nigrum	6572 Brachytic
2040 Canada Winter	3402 Gujarkhan	6991 Warrior
2261 Pusa	4821 Dorsett	7055 Titan
2276 Gatami	4823	
2284 Black Abyssinian	5899 Murasaki Mochi	C.P. 127422

Trebi and Cebada are similar in their disease and agronomic responses. Both are large-kerneled and have a tendency to be high in yield under dry-land conditions. Titan and Warrior very likely derived their resistance from the Trebi parent. Dorsett and C.I. 4823 are both black with brittle rachises and appear alike in many other respects. Brachytic and Murasaki Mochi are naked varieties and are not considered generally as good breeding stocks. Murasaki Mochi has a waxy endosperm. C.I. 2284 is classified as an Abyssinian intermediate type with black kernels. C.P. 127422 is a 2-rowed variety of German origin.

A large proportion of the selections being tested in the Wisconsin breeding program now have resistance to loose smut in one of the parents. Some of these selections are being tested for yields as well as disease reactions. Thus far the most promising source of resistance to loose smut has been Trebi. Unfortunately Trebi has a weak straw under Wisconsin conditions and most of its derivatives likewise have this undesirable agronomic characteristic. At present, efforts are being made to combine resistance to several major diseases with good agronomic type and smooth awns.

SUMMARY

Several methods of inoculating barley varieties with loose smut have been compared. The most effective and easily manipulated method consists of injecting dry spores into barley flowers by the use of a hypodermic needle to which is attached a small rubber bulb containing chlamydospores of *Ustilago nuda*. This is called the "needle" method and was used for testing varietal reaction. The reactions of 300 barley varieties from the C.I. collection are given. Wide varietal differences in percentage of infection were found. Several varieties possessed a high degree of resistance to the composite inoculum. Many selections from the breeding program have been tested for their response to *U. nuda*. Some of these are smooth-awned selections with loose-smut resistance and fairly good agronomic character-

istics. Efforts are being made to combine resistance to the several major barley diseases and good agronomic type with good malting quality.

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SEED TRANSMISSION OF BACTERIAL BLIGHT OF SUGAR BEET

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In the summer of 1943, the writers' attention was called to a disease in beet-seed fields in northern California and southern Oregon which involved a rapid dying of the leaf blades, commonly beginning at the tip of the blade and gradually becoming systemic. Later investigation of this trouble showed the disease was bacterial in nature. Although certain aspects of this disease have been described (1, 2, 3), the writers have observed some new features that are herewith discussed.

In 1909, Brown (1) described a bacterial disease of the leaves caused by *Phytomonas aptata*. The disease was observed by Townsend in Utah and California in 1908 (1). Brown and Jamieson (2), reporting on the causal bacterium of this disease of sugar beet from California, stated that "so far as the writers know, this organism has not been found in the field attacking the beet root, and, as none of the field beets with affected leaves had any root trouble, it is thought that the disease in the field is confined strictly to the leaf." Recently attention has been called by Carsner (3) to the occurrence of black leaf spot and stalk streak of sugar beet in the Pacific Northwest, caused by *Ph. aptata*.

The disease has been observed in moderate amounts in the Shasta Valley of northern California and in the Medford and Willamette Valleys of Oregon, at elevations above 4,000 feet where low temperatures occur frequently during the growing season. It is difficult to determine the amount of damage caused by bacterial blight because of complications induced by frost, insect injury, and other causes. The disease has not been observed in fields intended for sugar production although a few leaf infections were found on an overwintering test plot at Davis, California, planted with contaminated seed.

One of the striking symptoms of the disease in California and Oregon is the rapid dying of leaf blades. Under excessively moist conditions the dead parts become slimy and necrosis spreads quickly into the veins and the petioles of the leaf. Sometimes only isolated, black leaf spots are present. In other cases, necrosis begins in a wedge-shaped area near the margin of the leaf and spreads from there into the main veins and midrib, at times resulting in wilting of the leaf (Fig. 1, A and C). On the seed stalk, black streaks frequently extend from the tip to the base (Fig. 1, C). Petioles in the early phases of the disease have light to jet-black discoloration in the fibrovascular bundles, and the stalk, upon being split longitudinally, has dark cavities containing numerous bacteria (Fig. 1, D). The discoloration can usually be traced into the root where invasion was observed (Fig. 1, E). Invasion of the stalks causes brown to black discoloration of the spikelets and discoloration of the seedballs, which on culturing yield the pathogen.



FIG. 1. Bacterial blight of sugar beet. A. Sectorial infection at tip of the leaf. B. Infections on the cotyledons of sugar-beet seedlings from contaminated seed. C. Seed-stalk infection. Note black streaks along the stem extending from top to bottom. D. Longitudinal section of the stalk shown in C. E. Internal discoloration of a root infected with *Phytomonas aptata*. Natural infection. Bacteria were abundant in the discolored tissues. F. Healthy seedball. G. Infected seedballs (blackened).

Microscopic examination of diseased tissues revealed an abundance of bacteria. Inoculation tests with isolants from different parts of a diseased plant into young sugar beets growing in the greenhouse were successful in all cases. Symptoms typical of the disease were produced on leaf blades and petioles when they were inoculated by puncturing with a needle or by smearing the inoculum on the surface and rubbing with carborundum powder (6).

The bacterium isolated from various parts of the affected sugar-beet plants corresponds in all particulars with *Phytomonas aptata*, as described by Brown and Jamieson (2).

The bacteria seem to prefer the vascular elements of infected plants where they were found in much greater numbers and far in advance of those in the surrounding parenchyma.

Since the disease is not confined to the leaves but occurs in any or all parts of the plant, and may even cause death, it seems appropriate to apply a more descriptive and inclusive designation, namely, bacterial blight of sugar beet rather than leaf spot, or black streak, which are only phases of systemic infection.

Carsner (3) suggested, on the basis of his field observations, that the causal organism may be seed borne. The writers found usually not over 5 to 10 per cent infected seedlings from any lot grown in pasteurized soil. If seed from the same lot was germinated in Petri dishes, about 50 per cent of the seedlings were infected. Diseased seedballs can be detected by direct microscopic examination of sections made from them, by culturing, and by germination in Petri dishes or in sand. Such seedballs are noticeably darkened (Fig. 1, F and G) but similar darkening may also be induced by insect injury or other causes.

The first symptom in seedlings grown from contaminated seed (Fig. 1, B) is indicated by a small depression on either side of the cotyledons which becomes water soaked, sometimes developing a light-yellow halo. Gradually the affected parts of the cotyledons become soft and black. One to several lesions may occur on a single cotyledon, resulting in distortion. Seedlings grown in high humidity develop lesions on the true leaves. Thus, the pathogen can spread from infected cotyledons to other parts of a plant under favorable conditions of temperature and humidity.

Seedling infection was controlled in the greenhouse by treating field-contaminated seed with New Improved Ceresan (ethyl mercury phosphate) dust at the rate of $\frac{1}{2}$ oz. per 100 lb. of seed, New Improved Ceresan dip (1-1,200), and Arasan (50 per cent tetramethyl thiuram-disulphide) dust at the rate of $\frac{1}{4}$ oz. per 100 lb. of seed (Table 1). From these tests it is apparent that the disease is seed borne. However, no attempt was made to determine if the bacteria are primarily surface contaminants or if they are internal.

Brown and Jamieson (2) reported that *Phytomonas aptata* is capable of infecting the following plants by artificial inoculation: sweet or bell pepper

TABLE 1.—Seed treatments to control bacterial blight of sugar beet

Treatment	Healthy plants	Diseased plants	
	Number	Number	Per cent
Check, no treatment	176	11	5.9
	194	12	2.4
New Improved Ceresan,			
Dust	226	0	0
	381	0	0
Dip	270	0	0
	243	0	0
Arasan, dust	259	0	0
	272	0	0

(*Capsicum annuum* L.), kidney bean (*Phascolus vulgaris* L.), common eggplant (*Solanum melangena* L. var. *esculentum* Nees.), and lettuce (*Lactuca sativa* L.). Paine and Branfoot (5) in 1924 reported on a disease of lettuce which they first thought was caused by *Phytomonas marginalis* (Brown) Bergey *et al.*, but later they attributed it to *Ph. aptata*. It had affected 75 per cent of the lettuce plants in a private garden in England. The organism also caused a leaf spot on nasturtium (*Tropaeolum majus* L.) in nature according to Jamieson (4). The writers were able to produce the disease on all of these plants by needle inoculations with the isolants obtained from many diseased specimens of sugar beet. In addition, the broad bean (*Vicia faba* L.) was successfully inoculated with *Ph. aptata* which produced long black streaks on the stems of greenhouse-grown plants. When the inoculated plants were transferred to a moist chamber the damage was noticeably increased. Swiss chard (*Beta vulgaris* L. var. *cicla* L.) is also susceptible when the leaf blade or petiole is inoculated by needle.

SUMMARY

1. *Phytomonas aptata*, previously described as causing a leaf spot of sugar beet, is now known to produce blighting of the leaves, black streaking of seed stalks, and internal root necrosis.

2. Seed from blighted stalks are heavily contaminated with the bacterium and produce diseased plants upon germination. Seed transmission was prevented by using New Improved Ceresan dust or dip, or Arasan dust.

3. In addition to sugar beets, sweet pepper, nasturtium, lettuce, eggplant, and beans, previously reported to be susceptible to *Phytomonas aptata*, Swiss chard and broad bean have been infected.

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EFFECT OF MINERAL NUTRITION ON THE VIGOR AND SUSCEPTIBILITY TO BLIGHT OF OLD JAPANESE CHESTNUT TREES

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About 500 Japanese chestnut trees (*Castanea crenata* Sieb. and Zucc.) still survive from an original planting of 2,500 trees made 50 years ago near Fairfax Station, Virginia.² Most of these trees are in various stages of senility. It seemed desirable (1) to determine whether such trees could be benefited by the application of plant nutrients, and (2) to observe the effect of fertilization on incidence of infection and the susceptibility of the trees to the chestnut blight fungus, *Endothia parasitica* (Murr.) P. J. and A. W. Anderson.

In the spring of 1944 sixty-four of the surviving trees, of about equal vigor, were selected for treatment. They averaged 15.6 inches in diameter at breast height (d.b.h.), 33 feet in height, and 31 feet in crown spread. The experiment included seven fertilizer treatments and a check. Each treatment was replicated eight times in a randomized arrangement. Fertilizers were applied March 2, 1944, and a second application, of nitrogen only, to some of the trees was made on March 2, 1945. The potash and phosphate fertilizers were applied in "punch holes" 2 feet apart, and the nitrogen fertilizer was broadcast. Of the 64 trees, 26 were inoculated on 9 lower branches of each tree during the winter (December 19, 1944, to March 2, 1945) with 9 isolates of chestnut blight fungus from Orange, Massachusetts, and 9 from Bell, Maryland. One inoculation for each isolate of chestnut blight from each source and a check of sterile agar were placed on each branch. The cultures were isolated in October, 1944, from young cankers on American chestnut sprouts. Results of the inoculations were observed and d.b.h. and current shoot-growth measurements were made on July 20, 1945.

The various fertilizer treatments and the shoot lengths obtained are shown in table 1.

An examination of the data by means of the conventional analysis of variance indicates the following conclusions: (1) Fertilization with potash and phosphoric acid had no significant effect on shoot growth. (2) Nitrogen at the rate of 150 lb. was without significant effect. (3) Nitrogen at the rate of 300 lb. increased the shoot growth significantly in 1944, but the second application, in 1945, had no significant effect.

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Under the conditions of this test no fertilization except a very heavy application of nitrogen had any significant effect. Shoot growth on the trees fertilized with potash and phosphoric acid was not significantly greater than on the nonfertilized trees. The data do not permit statistical evaluation of the effects of climatic differences in the two years.

There is no correlation between shoot length and trunk diameter in the trees in this experiment.

The results of the canker inoculations are more difficult to interpret than are those of the shoot length. One Massachusetts and one Maryland isolate of the fungus produced no cankers at all while other isolates of each group produced only two to three. Some of the trees were susceptible, as evidenced by the cankers that developed following inoculation, and others appeared

TABLE 1.—*Influence of fertilizer treatment on current shoot growth of 50-year-old Japanese chestnuts*

Treatment No.	Fertilization, ^b lb./acre	Ave. total shoot length per tree ^a		
		1944	1945	Both years
		cm.	cm.	cm.
1	None	175.6	201.3	376.9
2	300 lb. N annually	218.6	225.8	444.4
3	150 lb. N annually	192.8	225.4	418.1
4	300 lb. N once	223.1	226.0	449.1
5	300 lb. N once + P-K in 1944	199.8	234.0	433.8
6	150 lb. N annually + P-K in 1944	194.1	217.4	411.5
7	300 lb. N annually + P-K in 1944	205.4	226.8	432.1
8	P-K in 1944	162.5	187.8	350.2

^a Average total length of 10 shoots per tree, one tree per replicate, 8 replicates.

^b Nitrogen applied as ammonium nitrate. P-K (100 lb. P_2O_5 plus 75 lb. K_2O) applied as superphosphate and KCl, respectively.

to be entirely resistant. The data obtained are thus scattering, with a great many zero values. This renders statistical treatment difficult and of doubtful value. Nevertheless some of the differences in number and area of cankers per tree are so large that there can be little doubt of their true significance. Table 2 presents the average number of cankers and canker area per tree for each fertilizer treatment.

When P-K was applied without nitrogen, Treatment 8 (all of the P-K treatments were applied a year before inoculation), the incidence and susceptibility to canker, as measured by the number and area of cankers per tree, respectively, were both much greater than with all but one of the treatments that consisted of nitrogen only. This exception occurred where 300 lb. of nitrogen were applied annually (Treatment 2) in conjunction with the Maryland blight isolates. On the Massachusetts isolates this treatment did not appear to differ significantly from the other treatments with nitrogen only. But when nitrogen was applied in conjunction with P-K at the lower rate annually, or once at the 300-lb. rate (Treatments 5 and 6, respectively), it appears to have rendered the trees less susceptible. When applied again

at 300 lb. per acre near the time of inoculation it failed to give this result. This observation appears to correlate with the observation that susceptibility increased when 300 lb. of nitrogen without P-K were applied annually. With the exception just noted, nitrogen treatments without P-K, whether made once or annually, had no significant effect on canker incidence or area of cankers.

The average size of the cankers varied, but in a manner that appears to have been random; there seems to have been no connection between canker size and the fertilizer treatments in this experiment. There was also no apparent significant difference between the Massachusetts and Maryland isolates in the average size of canker produced (4.2 and 4.5 sq. cm., respec-

TABLE 2.—*Effect of fertilization on occurrence and development of blight cankers induced by inoculation of 50-year-old Japanese chestnut trees*

Treatment No.	Fertilization, lb./acre	No. of trees	Av. No. of cankers per tree	Av. canker area (sq. cm.) per tree
Endothia isolates from Massachusetts				
1	None	3	1.0	2.4
2	300 lb. N annually	3	1.6	6.8
3	150 lb. N annually	2	1.5	6.9
4	300 lb. N once	4	1.0	5.5
5	300 lb. N once + P-K in 1944	3	1.7	5.4
6	150 lb. N annually + P-K in 1944	4	2.5	5.7
7	300 lb. N annually + P-K in 1944	3	3.3	19.1
8	P-K in 1944	4	3.0	14.2
Endothia isolates from Maryland				
1	None	3	1.0	2.8
2	300 lb. N annually	3	2.0	12.7
3	150 lb. N annually	2	0.5	2.1
4	300 lb. N once	4	0.8	3.4
5	300 lb. N once + P-K in 1944	3	0.7	2.1
6	150 lb. N annually + P-K in 1944	4	2.2	4.9
7	300 lb. N annually + P-K in 1944	3	3.3	10.3
8	P-K in 1944	4	3.0	14.9

tively) or in the total number of cankers produced by each (52 and 46, respectively). There is thus no evidence, from this experiment, that the longer established group of Massachusetts isolates are any less virulent than the Maryland isolates that appeared more than 10 years later.

It is clear that, under the conditions of this experiment, P-K applied a year previous to inoculation increased canker incidence and susceptibility to the canker fungus, but this effect was modified in a complicated manner by treatment with nitrogen. These results are of sufficient importance to warrant further study. Field observations made elsewhere during six growing seasons suggest that potassium chloride is an unfavorable form of potash for chestnuts. The current observations may thus be connected with some toxic action of the chloride ion.

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RUST AND PHOSPHORUS DISTRIBUTION IN WHEAT LEAVES

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Despite the many years which have elapsed since the early work of Marshall Ward (13 and 14) on the physiology of obligate parasitism, this complex is still in many respects an enigma. One of the phenomena which has been fairly well established is that the rusts and the powdery mildews increase the respiration of their hosts. As early as 1915 Reed and Crabill (12) demonstrated that apple leaves infected with *Gymnosporangium juniperi-virginianae* respire more than noninfected leaves. Yarwood (15 and 16) showed that a similar change takes place when excised clover leaves are inoculated with either rust or mildew. Pratt (11) found that powdery mildew increases the respiration in wheat plants and also that the higher rate is not due to the respiration of the mildew mycelium. More recently Allen (1, 2, 3) has shown that in the powdery mildew of wheat the increase in the respiration and metabolism occurs during the period of rapid growth and maturation of the parasitic colony and that a decline sets in after this period. Associated with these phenomena is an accumulation followed by a decrease in the quantity of carbohydrates. This increased metabolism is mainly due to the oxidation of glucose. The importance of carbohydrate metabolism in the physiology of obligate parasitism is then evident.

Some of the vital factors in the degradation of glucose are the phosphate transfer enzymes, and James and Bunting (7) have shown that such enzymes are also operative in barley, a host of stem rust. The importance of such phosphorus enzymes suggests the possibility that an accumulation of phosphorus may be linked to the high rate of utilization of the sugars, so that an increase in phosphorus would allow greater enzyme transfer and a more rapid oxidation and respiration. Humphrey and Dufrenoy (6) have indicated that in oats infected with crown rust, phosphorus is released by infected cells and made available to the parasite, and they postulate a transfer of phosphorus into the infected regions. Little more is known of the phosphorus relations of infected leaves.

The current investigations had two objectives: (1) the development of a technique for the study of phosphorus relationships, and (2) a study of the distribution of phosphorus in normal leaves and rust infected leaves.

METHODS

The paucity of information on the physiology of obligate parasitism is due in large part to the fact that no means of culturing an obligately parasitic fungus *in vitro* has been known. An investigator could obtain and study only the spores of the parasite apart from the host plant. Any re-

¹ These studies were conducted while the senior author was on the staff of the University of Delaware.

search must therefore deal with activity of the pathogen at work within its host. Humphrey and Dufrenoy (6) resolved this difficulty by the use of staining techniques, but even this method had severe limitations. Quantitative data are difficult to obtain, and observations on the density of stain cannot be easily reproduced. To overcome such difficulties, a tagged element, radiophosphorus, was used in these investigations. The use of radioactive phosphorus enables the investigator to detect very small quantities of the element, for the assay is dependent upon the disintegration of the atom, and theoretically the presence of each atom can therefore be recorded. The method is relatively simple because no complex chemical separations are necessary. Finally, radiographs can pictorially locate any differences in concentration of the element in various parts of the host. As far as is known, the radioactive phosphorus, P^{32} , is metabolized by the living organism in a manner similar to the common form of phosphorus (5) and its biologic activity is generally accepted as representative of the normal isomer.

Only one host, Little Club wheat, and one pathogen, *Puccinia graminis tritici* race 34, were employed in these experiments.² The radioactive phosphorus, P^{32} , was obtained from the Massachusetts Institute of Technology³ as phosphoric acid and converted to KH_2PO_4 for these experiments. Hoagland's complete nutrient solution (9) was the basic medium for growing the wheat seedlings, with but one change, the substitution of the radioactive phosphorus salt for the usual form. A Geiger counter (1) was used for the analysis of phosphorus, and ordinary Verichrome film served for the radiographs. The Geiger counter⁴ was mounted 0.5 cm. above the material to be analyzed.

Wheat seeds were disinfected with mercuric chloride and allowed to germinate and grow in a moist chamber until the first true leaves just ruptured the coleoptiles. The seedlings were then transferred to the nutrient solution contained in small circular glass dishes, 6 inches in diameter and 3 inches high, which contained $\frac{1}{4}$ -inch mesh wire for support of the plants. When the plants were about 100 mm. tall, one side of the distal half of each leaf was inoculated with the rust by brushing the leaves with a suspension of urediospores in water; the plants were then placed in a moist chamber for two days. An equal number of check plants was treated similarly except that they received no inoculum. The plants were kept in a constant temperature room at $20^\circ \pm 2^\circ$ C. under a 200-watt Mazda lamp during the entire experiment. Only the first true leaf was inoculated and all other leaves were clipped off as soon as they appeared. Under these conditions, flecks first were observed 4 days after inoculation, and sporulation of the

² The wheat wa. furnished by Dr. C. S. Holton of the United States Department of Agriculture and the rust by Dr. H. A. Rodenhiser of the United States Department of Agriculture.

³ The radioactive phosphorus was obtained through the courtesy of Dr. R. D. Evans of the Massachusetts Institute of Technology.

⁴ The counting equipment was obtained from Dr. A. J. Allen, University of Pittsburgh.

rust fungus occurred 10 days after inoculation. No additional nutrient solution was added to the dishes until the fifth day after inoculation. From then on, sufficient solution was added every two days to bring it to the original volume. Radioactive phosphorus was present only in the original medium, and the normal inactive salt in all the later solutions.

Four plants were used for each analysis with the Geiger counter and three replicate analyses were made 2, 5, and 12 days after inoculation. The leaves were used either whole or cut into two sections, the infected distal end and the noninfected basal portion. Each leaf section was further divided into $\frac{1}{4}$ -inch sections to facilitate digestion. These tissues were dried at 90° C. for 8 hours and their dry weights determined. Digestion was carried out by adding 20 ml. of concentrated nitric acid to the leaves and the mixture heated on a water bath for two hours. This treatment completely dissolved the tissues. Enough water was then added to bring the total volume again to 20 ml. An aliquot of 0.2 ml. was placed in the standard depression slide and heated at 56° C. until dry. Uniform films were thus obtained. Treatment of the urediospores was similar to that of the leaves with few modifications. Leaves of the 150 wheat seedlings were shaken over an evaporating dish and the urediospores collected by brushing them directly into drying dishes. After drying, the spores were found to be resistant to complete digestion with nitric acid, and aqua regia was used in its stead. Three such slides were made for each determination. All results recorded were obtained by averaging the counts from the three aliquots.

For the actual determinations a small Geiger tube, sensitive to beta rays, was mounted in a wooden frame so that when the slides were placed beneath it, the plane surface of the slide was 0.5 cm. from the tube. Twenty-minute background counts were made before each day's analyses, and all counts on the tissue digests were made for 10 minutes. Preliminary studies with longer exposures showed no gain in accuracy. Because radioactive phosphorus continuously and irrevocably disintegrates, corrections for the time interval between the clipping of the leaves and counting were made from a decay curve (5). The active phosphorus was recorded as counts per gram dry weight, to which it is directly proportional (5). All figures have been rounded off to the third digit, because in these analyses only differences in the first two digits were important.

To follow the changes in the distribution of phosphorus, radiographs (5) of the leaves were made every second or third day after inoculation of the seedlings. Fresh green leaves were mounted on glass plates with transparent cellulose tape, through which the beta rays from the P^{32} can pass. This type of mount was easy to handle and also preserved the leaf in a green condition for regular photographs. Verichrome film was placed against the mount, and it in turn was covered with a glass plate.

ABSORPTION AND DISTRIBUTION OF PHOSPHORUS

The infection of leaves of Little Club wheat with *Puccinia graminis tritici* does not appear to affect the total amount of phosphorus in the leaves.

TABLE 1.—*Concentration of radioactive phosphorus in inoculated and noninoculated leaves*

No. days after inoculation	Counts of radioactivity ^a	
	Inoculated leaves	Noninoculated leaves
2	16,400	22,100
5	24,500	17,800
10	22,300	21,300
Average	21,067	20,400

^a Counts were recorded on the basis of counts per gram dry weight.

A comparison of the radioactive phosphorus in the whole leaves of inoculated and noninoculated plants reveals no consistent differences in the amount of that element (Table 1). When all replicates were averaged, however, a slightly greater concentration was found in the inoculated seedlings.

The total amount of phosphorus absorbed by the plant was not significantly influenced by the disease but its distribution within the leaf was affected. More phosphorus was present in the distal parts of all leaves than in the basal parts. The difference in concentration between the two parts of the leaf was, however, much greater in inoculated plants than in the check plants. This increased accumulation in the infected distal portion was consistently greater in all the replicates, even though the phosphorus determinations were made at intervals from the second to tenth day after inoculation. On the average, the difference in phosphorus counts between the distal and basal leaf sections were twice as great for the seedlings in which the upper part was infected as for the normal plants; the differences were 4,667 and 1,967, respectively (Table 2).

Because of the possible presence of extraneous radioelements which would confuse the results, analyses were made on leaves grown in the normal Hoagland's solution. No radioactivity was detected in these leaves. Therefore, all measured activity in the leaves was due to the beta rays emitted by the P³².

To study the reason for the increased amount of phosphorus in the infected portion of the leaf, radiographs were made at various intervals of

TABLE 2.—*Distribution of radioactive phosphorus in leaves*

No. days after inoculation	Counts of radioactivity					
	Inoculated leaves ^a			Noninoculated leaves		
	Distal sections	Basal sections	Difference	Distal sections	Basal sections	Difference
2	18,700	14,100	4,600	23,400	20,700	2,700
5	27,000	22,100	4,900	18,500	17,300	1,200
12	24,500	20,000	4,500	22,300	20,300	2,000
Average	23,400	18,733	4,667	21,400	19,433	1,967

^a Distal sections were infected with rust, basal sections were not infected.

time after inoculation. Film was exposed to leaves clipped from the plants just before inoculation and at 2, 4, 5, 6, 7, 8, 9, and 10 days after inoculation. Flecking was first observed on the fourth day, raised pustules on the eighth

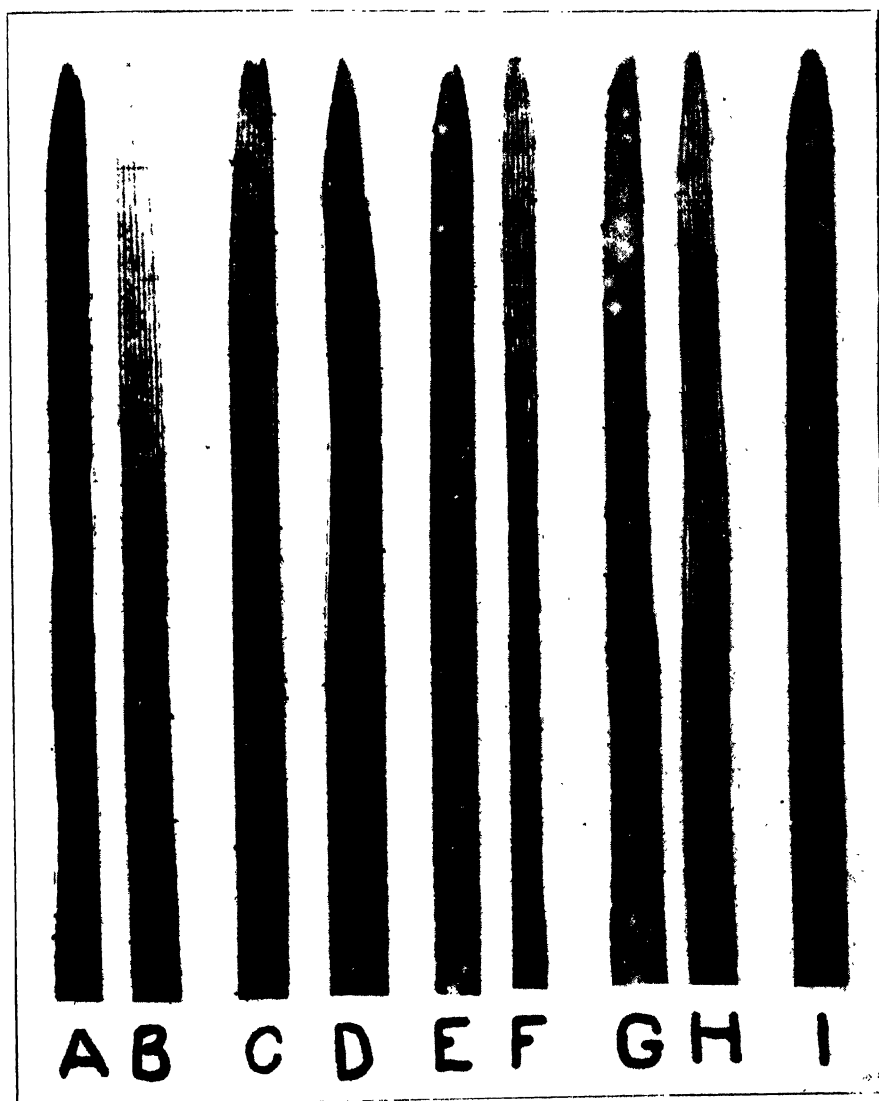


FIG. 1. Radiographs and photographs of *Puccinia graminis tritici* on Little Club wheat taken on various days after inoculation. A and B. Radiograph and photograph, 2nd day. C. Photograph, 4th day. D. Radiograph, 5th day. E and F. Radiograph and photograph, 6th day. G and H. Radiograph and photograph, 7th day. I. Radiograph, 8th day.

day, and sporulation on the ninth day after inoculation. In radiographs made on the second day after inoculation phosphorus was evenly distributed in the leaf. On the fourth day, coincident with the period when flecks were

becoming visible, differences in the density of the negative were observed. At this time the differences in density of these areas of the leaf were not sufficient for good reproduction. From then on, as the flecks became further differentiated, the density of the film in the infection court increased until the ninth day when the spores began to rupture the epidermis (Fig. 1, D, E, G, and I).

Photographs were made of the leaves that were exposed for the radiographs. Some of these are reproduced in figure 1, B, C, F, and H. A comparison of radiographs and photographs showed that the phosphorus is concentrated in the infected areas of the leaf. It is apparent then that rust infection stimulates the accumulation of radioactive phosphorus in the diseased areas of the plant. That the images of the leaves and infected areas are due to the radioactive phosphorus was demonstrated by placing two leaves side by side, one from a seedling in a nutrient solution containing radioactive phosphorus and the other containing normal phosphorus. Only the leaf containing the radioactive material affected the film and left an image on the negative. The entire series of plants for the radioactive studies were rerun and similar results were obtained.

The accumulation of unusually large quantities of phosphorus in the infection court might be due to two causes; either phosphorus is deposited in the urediospores or mycelium of the fungus, or in the infected cells of the host. Because of the difficulties in separating the host tissues from the mycelium of the parasite in quantity large enough for analysis, no determinations could be made on the components of this complex. However, the urediospores were collected and analyzed. Radiographs of the spores indicated the presence of P^{32} . The specific activity of phosphorus contained in the spores was less than the mean average of both infected portions of leaves and of entire healthy leaves. The specific activity of the spores was 17,200, for the entire noninoculated leaves it was 20,400, and for rusted leaves 21,067. We can assume, therefore, that the increased accumulation of phosphorus in the infected areas of the plant was not in spores but might be in infected host cells. Another possibility does still exist, namely, that the accumulation is in the mycelium of the rust.

DISCUSSION

The effect of *Puccinia graminis tritici* on the phosphorus distribution in infected wheat leaves is similar to the effect of rusts and mildews on the plant carbohydrates: in both cases the materials are concentrated in the invaded areas of infected leaves (3, 12). If studies from both obligate parasites, the rust and the mildew fungi, can be considered as complementary, a picture of some of the physiological responses in obligate parasitism can be reasonably formulated. The pathogen is dependent upon its host for carbohydrates. Any unfavorable conditions such as light, temperature, and nutrition, which prevent normal plant vigor and synthesis of carbohydrates, reduce the supply of sugars and also the virility of the pathogen

on its host. As a reaction to the increased nutritive demands of the parasite, sugars and phosphorus accumulate in the infected areas of the leaves. The augmented phosphorus supply might permit a greater activity of the phosphorylating enzymes of intermediary sugar metabolism. Thus the increased amount of carbohydrates would be consumed and respiration consequently increased. Eventually the demands upon the host's cells are too drastic or some toxic action ensues, and necrosis occurs.

Unfortunately, it is not known whether the concentration of phosphorus and carbohydrates is in the fungus mycelium or in the host cells; in either case an increased respiration of the infected tissues would result. Humphrey and Dufrenoy (6) indicated that the mycelium receives its phosphorus from the invaded cells and that the spores are "rich in phosphorus." However, analyses of urediospores revealed no such undue accumulation, for less radioactive phosphorus per gram dry weight was found in the spores than in either infected or normal leaves. It is plausible to conclude that the concentration of phosphorus might be in the infected host cells. Whether the increased amount of phosphorus in rusted areas is due to a specific action of the parasite or is merely a general reaction of plant cells to injury and irritation is still undetermined. However, studies of injured animal tissue indicate that, even without the presence of a parasite, phosphorus compounds are sometimes secreted into the damaged areas (4). Yeast cells also secrete the phosphorus compounds when subject to mild irradiation with X-rays (8). Until further studies are made, any interpretation of the cause for the concentration of phosphorus in tissue infected by obligate parasites must be accepted with reservation.

SUMMARY

1. No consistent differences in total content of radiophosphorus were found between entire rusted and healthy wheat leaves.
2. In inoculated leaves the differences in phosphorus content between the infected distal portion and the noninfected basal portion of the leaves were much greater than the differences of similar areas in healthy plants. The distal parts always contained more phosphorus.
3. The phosphorus was accumulated in rusted parts of leaves and concentrated in the areas invaded by the parasite.
4. The concentration of radiophosphorus in the urediospores was less than in the leaves.

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DIPLODIA DIE-BACK OF GUAYULE (PARTHENIUM ARGENTATUM GRAY)

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INTRODUCTION

A die-back of guayule caused by *Diplodia theobromae* (*D. natalensis*) (Pat.) Nowell,² occurred in southern Texas during the summer of 1944, and was first observed following the summer rains. In two-year-old irrigated plantings, where the crowded condition of the plants favored the disease, practically every plant was infected and many plants were dead by mid-October. In dry-land plantings and in one-year-old irrigated plantings infections were few and there was relatively little damage from the disease.³ Dry weather retarded the disease and during the winter fungus activity ceased entirely. With the return of high temperatures and rain in the spring of 1945 many of the old lesions again became active. In addition to the old lesions, the pycnidia which were produced in abundance on the diseased plants furnished abundant inoculum for new primary infections.

SYMPTOMS

Infection generally starts in the leaves or small twigs and progresses downward. In the field the leaf infections begin as irregular necrotic lesions which soon coalesce. The leaves die, generally from the tip, with pronounced yellowing of the leaf after one-half of the blade has been killed. Infections that begin on small twigs soon progress downward and involve the larger branches. When the lesion has nearly girdled a large branch the leaves become yellow and the diseased branch is very conspicuous among healthy foliage. As soon as the branch is completely girdled it dies (Fig. 1, C). When infection occurs near the ground line the entire plant succumbs as soon as it is girdled by the fungus lesion. After the plant or branch dies, provided moisture conditions are favorable, black pycnidia of the fungus develop under the diseased bark and erupt as elongated black carbonaceous masses (Fig. 1, D).

INOCULATIONS

Methods

Experiments were conducted at Salinas, California, to determine the effects of temperature, moisture, and age of plant on infection by *Diplodia*. Pycnidial material for the experiment was collected from diseased plants in Texas in October, 1944. A spore suspension was obtained by soaking

¹ Formerly Pathologist, Special Guayule Research Project, U. S. Department of Agriculture.

² Specific determination of the organism was made by John A. Stevenson, Division of Mycology and Disease Survey, U. S. Department of Agriculture.

³ Presley, John T. *Diplodia* die back of guayule. U. S. Dept. Agr., Plant Dis. Repr. 29: 64. 1945.

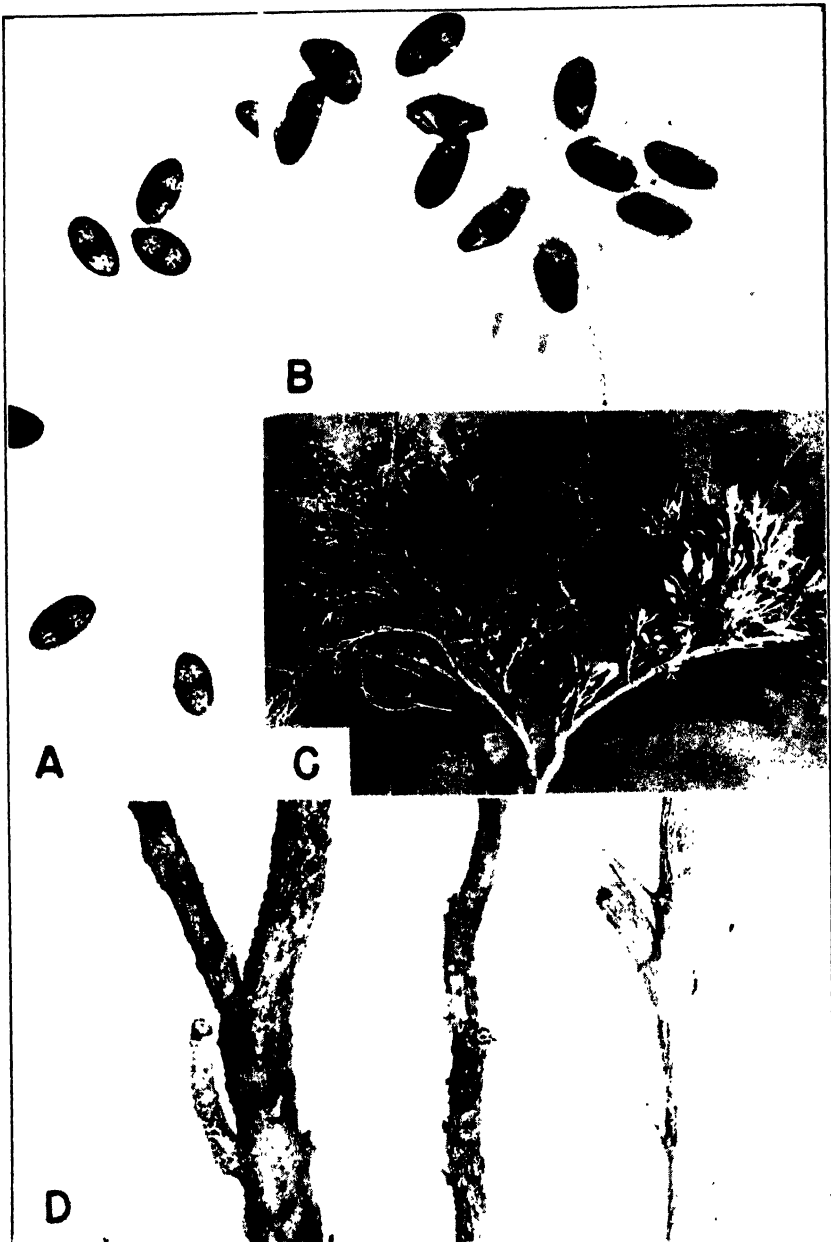


FIG. 1. *Diplodia thiebromae*. A, spores; B, germinating spores; C, 2-year-old guayule plant with one diseased branch; and D, pycnidia on two diseased branches on left, healthy branch on right.

and agitating the pycnidial material in flasks of distilled water. The guayule plant material and seed used in the experiments were of the standard variety No. 593, and were placed in temperature tanks at the beginning of the experiment.

The first experiment consisted of three treatments: (1) Guayule seed, 100 in each pot, were planted in pots of pasteurized soil, and the soil surface sprayed with a suspension of *Diplodia* spores. (2) The leaves of transplanted nursery stock that had been permitted to grow for six weeks in 8-inch pots were punctured to simulate insect injury and sprayed with the spore suspension. (3) The noninjured leaves of similar transplanted nursery stock were sprayed with the spore suspension.

Plants of two different ages were used in the second experiment. The first consisted of transplanted nursery seedlings that had grown for eight weeks in the greenhouse and had reached the flowering stage, but with all of the leaves still green and active. The second consisted of older plants that had grown in pots outside the greenhouse for many months. These plants were in a hardened condition and had many dead leaves on the lower portions of the stems, and they were very similar to field-grown guayule.

All plants were sprayed with the spore suspension, placed in temperature tanks, and the containers covered to maintain a high relative humidity around the plants. The plants were then sprayed with tap water at one-hour intervals through the day to insure a film of water on the leaves. At the end of 24 hours four containers at each temperature had the covers removed. These and the covered plants were sprayed at intervals throughout the day with water to keep them moist. After 48 hours a second series of four covers was removed and all of the plants sprayed throughout the day as before. An additional series had the covers removed after 72 hours and 96 hours.

Three inoculated and one check plant for each treatment were used at each temperature, ranging from 50° to 100° F. in 10° increments. All plants were placed in the temperature tanks and allowed to stand for 12 hours for temperature adjustment before being sprayed with the spore suspension. A small hand sprayer was used and each plant was sprayed until thoroughly wet with the spore suspension. Subsequently the plants were watered as needed to keep them vigorous.

A germination test of the fungus spores proved them to be viable (Fig. 1, A and B). Dry slides covered with spores were placed in moist chambers where the humidity was maintained at 100 per cent; also, slides with spores in drops of distilled water were kept in moist chambers. The spores in the drops of water germinated after about four hours at room temperature (70° to 80°) (Fig. 1, B), but it required almost a full day for germination of the spores on the dry slides maintained at 100 per cent humidity.

Results

Experiment 1, Treatment 1. The plants began emerging after four days at 80° and 90° F., two to three days later at 60°, 70°, and 100°, and after two weeks in the 50° tank. Soon after emergence some of the seedlings at 70°, 80°, and 90° had symptoms of damping-off. Four seedlings damped-off at 70°, 4 at 80°, and 5 at 90° F. *Diplodia* was isolated from all the diseased

seedlings. Counts of emerged seedlings after 14 days at the various temperatures demonstrated that *Diplodia* caused appreciable loss only at 80° F. (Table 1).

Experiment 1, Treatments 2 and 3. Three days after being sprayed with the spore suspension there was no apparent infection of the plants at any temperature. These plants were again sprayed with the spore suspension on March 10 and half of the plants in both treatments were covered in order to hold the humidity at a high level and prevent drying of the film of water on the leaves. On March 12 some of the covered plants at 80°, 90°, and 100° F. had symptoms of *Diplodia* infection on the leaves and small twigs.

Isolations were made from the visibly infected twigs and *Diplodia* was recovered from all of the platings. Isolations were also attempted from twigs without visible infection symptoms. For all of these platings, a por-

TABLE 1.—*Emerged guayule seedlings after 14 days in soil infested with Diplodia, at various temperatures*

Soil temperature	Emerged seedlings, inoculated soil	Check
	Average of 3 replicates	
<i>Degrees F.</i>	<i>Number</i>	<i>Number</i>
50	12	16
60	47	41
70	39	46
80	26	43
90	29	31
100	16	16

tion of the twigs was surface sterilized for two minutes in commercial Clorox, full strength; the remaining portion was not surface sterilized but was washed thoroughly in distilled water and plated. In the higher temperature series, *Diplodia* was recovered from both surface-sterilized and non-surface-sterilized material, indicating that the fungus had penetrated the plant tissue. *Diplodia* was recovered from the non-surface-sterilized but not from the surface-sterilized material at 50° F., indicating that inoculum was present in a viable condition, but the temperature was not favorable for infection to take place. There appeared to be no difference in amount of infection between treatment 2 and treatment 3. This test established the pathogenicity of *Diplodia* on guayule at certain temperatures, hence an additional test was needed to establish the moisture-time relationship of the infection.

Experiment 2. Final observations were made on March 26 (six days after spore suspension was applied) and photographs taken of the plants that had been covered for 48 hours (Fig. 2). The results at the various temperatures are given in table 2.

Plants from the 100° F. tanks were not included in the photographs because of the complete break-down of the leaves on both young and old plants at that temperature when covered for two days or more. The high humidity

and high temperature acting together apparently were responsible for the break-down. In the first experiment the uncovered check plants at 100° F. survived, hence the covering at 100° seems to have had some effect other than aiding fungus penetration.

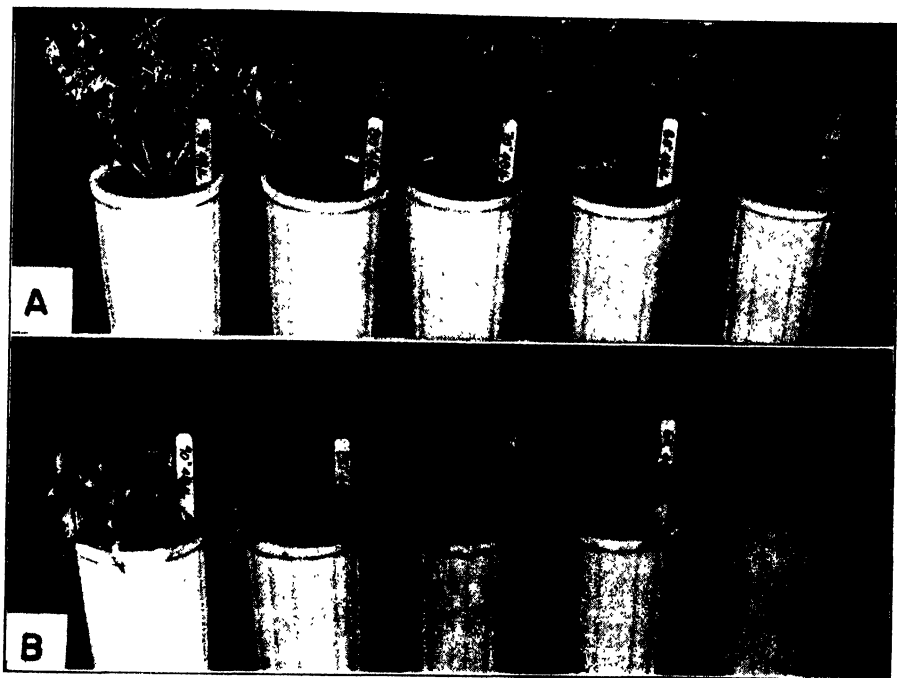


FIG. 2. Guayule inoculated with *Diplodia theobromae*. A, effect of induced disease on hardened plants at indicated temperatures and time intervals. B, effect of inoculations on young succulent plants at indicated temperatures and time intervals.

DISCUSSION

The inoculation tests established the fact that a high relative humidity and high air temperatures are necessary for rapid infection by *Diplodia*. Infection takes place more quickly in older hardened plants than in younger vigorously growing plants. Dead leaves on the plants definitely favor infection, perhaps because the fungus grows saprophytically for a time on the dead leaves and then penetrates the green tissue. The rate of decline of infected plants is also markedly influenced by temperature and humidity. At all temperatures up to and including 90° F. there was no apparent fungus injury to the plants during the first 24-hour period when they were covered. At 48 hours and subsequently, there was definite fungus injury at temperatures above 60°; hence it may be concluded that in an area such as south Texas during the rainy period in late summer when temperatures are high, the rains frequent, and the relative humidity high, infection by *Diplodia* will take place. Optimum conditions for infection do not occur every summer as evidenced by the fact that guayule plantings had been in south Texas

TABLE 2.—*Infection of guayule plants by Diplodia as influenced by humidity at various temperatures*

Days covered	Temperature	Young plants	Old plants
No.	° F.		
1	50	No visible symptom	No visible symptom
2		do	do
3		do	do
4		do	do
1	60	do	do
2		do	do
3		do	Slight damage to older leaves
4		do	Tips of healthy leaves slightly affected and older leaves more so
1	70	do	Slight damage to healthy leaves
2		do	Pronounced damage to the leaves and some twig infection
3		Necrotic areas on most of the lower leaves	Pronounced damage to the leaves and some twig infection
4		Necrotic areas on most of the lower leaves	Pronounced damage to the leaves and some twig infection
1	80	No visible symptom	No visible symptom
2		Slight necrosis on lower leaves	Definite infection of stems and pronounced damage to the leaves
3		Pronounced necrosis on lower leaves	1 plant dead and 1 partly dead
4		Practically all leaves severely affected and dying	1 plant dead and 1 partly dead
1	90	Slight necrosis of lower leaves	No visible symptom
2		Necrosis more pronounced	1 plant completely dead, 1 partly dead
3		Rather general infection of leaves except at growing tip of plant	Both plants dead
4		1 plant dead, other severely affected, most of the leaves dead	1 plant dead, other severely affected
1	100	1 plant very slightly necrotic, other severely damaged	Few dead twigs and extensive leaf injury
2 ^a		All leaves dead when uncovered	Plants severely affected, many twigs dead
3		All leaves dead when uncovered	Both plants dead
4		All leaves dead when uncovered	Both plants dead

^a The complete breakdown of the leaves on both old and young plants when covered for 2 days or more at 100° apparently resulted from factors other than fungus invasion.

more than two years before an outbreak of *Diplodia* of sufficient severity to cause appreciable damage was recognized.

The closely related species *Parthenium incanum* H.B.K., known as mariola, is also susceptible to *Diplodia*. The infection was evidenced by pronounced spotting of the leaves but no branches or twigs were killed on the plants inoculated. It may be assumed that under the conditions of the experiment mariola is less susceptible to *Diplodia* infection than guayule.

SUMMARY

Diplodia theobromae caused a severe die-back of 2-year-old irrigated guayule in south Texas in late summer and early fall of 1944. Younger

irrigated shrub and 2-year-old shrub on dry-land plantings were not appreciably affected.

Inoculation experiments demonstrated that spores in the soil could cause the loss of small seedlings, especially at 80° F. Leaf and twig infections in the greenhouse were dependent upon high humidity for 24 or more hours and temperatures of from 80° to 90° F. Hardened plants with old dry leaves were more susceptible to infection than young succulent plants with green active leaves.

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PHYTOPATHOLOGICAL NOTE

Copper 8-Quinolinolate, a Promising Fungicide.—Preliminary laboratory and field tests have indicated the possible use of an organic compound for the control of plant diseases. In glass-slide fungicide tests the compound 8-quinolinol and its copper derivative were both highly toxic to *Sclerotinia fructicola*, having an L.D.₅₀ range of less than 1.5 micrograms per sq. cm. of glass slide. Chlorine substitution on the 5 and 7 positions of these parent compounds decreased toxicity to an L.D.₅₀ of 50 and 200 micrograms per sq. cm. of glass slide, respectively, while bromine substitutions in the same positions decreased toxicity to an L.D.₅₀ of 400 and 1200 micrograms, respectively.

In 1945 these materials were used in field tests for the control of apple scab and blotch. Other materials used for comparison were Puratized N5-E (10 per cent phenyl mercuri triethanol ammonium lactate), Isothan Q 15 (20 per cent lauryl isoquinolinium bromide), Isothan Q 32 (20 per cent cetyl isoquinolinium bromide), and Fermate (ferrie dimethyldithiocarbamate).

A 30-year-old block of Duchess apple trees was divided into single-tree blocks with three single-tree replications per treatment. Spray applications were made on April 27, May 9 and 23, June 1, 11, and 22, and July 11. The first spray, April 27, was at the early bloom period and contained only the respective fungicide treatments. The five succeeding applications contained the fungicide plus 3 lb. of lead arsenate and 3 lb. of hydrated lime to 100 gallons of water, except that lime was omitted from sprays containing Fermate. Fermate in itself is an arsenical safener. The other fungicide treatments are not known to be arsenical correctives, thus lime was added whenever lead arsenate was used with them. The last application on July 11, contained only the fungicide treatment. The amount of each fungicide used to 100 gallons of spray is shown in table 1. Final fruit records were taken on July 25 (Table 1).

Although 8-quinolinol and its copper derivative were both highly toxic in the laboratory, the former did not retain its toxicity in the field and was the least effective of the six materials tested. This might possibly be explained by its higher solubility in water, which would prevent less residual toxicity to be retained. The copper content of the latter may have increased its effectiveness in the field over the parent compound. Copper 8-quinolinolate, was highly fungicidal and compared favorably with Fermate and Puratized N5-E in controlling scab and blotch. Commercial control was not secured with Isothan Q 15 and Q 32.

Under the conditions of the trial there was no visible fruit or foliage injury from any of the six materials tested. Fruit analysis (Table 1) of acid and soluble solids indicated that the fungicidal treatment affected indirectly the maturation. This fruit maturation difference was apparently directly correlated with fungus infection in that the more severe the infection, or the less the control, the higher was the degree of maturation.

TABLE 1.—*Efficacy of 8-quinolinol and its copper derivative in the control of scab and blotch on Duchess apple in 1945*

Treatment	Amount to 100 gal. of water	Fruit analyses			Percentage of fruit infected	
		Per cent acid	Per cent soluble solids	Degree of matur- ation ^a	Scab	Blotch
Fermate	1 lb.	1.38	8.7	M 2	37.8	0.1
Cu 8-quinolinolate	1 lb.	1.31	8.7	M 3	17.3	0.7
Puritized N5-E	$\frac{1}{2}$ pt.	1.50	8.7	M 1	11.1	7.9
Isothan Q 15	1 pt.	1.31	9.4	M 3	38.9	54.0
Isothan Q 32	1 pt.	1.32	10.0	M 3	42.3	66.1
8-quinolinol	1 lb.	1.20	9.3	M 4	40.7	86.2

^a M 1, M 2, M 3, M 4—advancing degrees of maturation.

The compound copper 8-quinolinolate shows considerable promise as a protectant for apple scab and blotch.

The writer wishes to express appreciation to Dr. R. V. Lott, Associate Professor of Pomology in the Department for making the fruit analyses.—
DWIGHT POWELL, University of Illinois, Urbana, Illinois.

REPORT OF THE 37TH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The American Phytopathological Society held its 37th annual meeting with the American Association for the Advancement of Science in St. Louis, Missouri, March 27-30, 1946. The sectional and business sessions were held in the Jefferson and Statler hotels. Approximately 325 members attended. Eighty-six papers on original research were accepted by the editorial committee for presentation at the meeting. The sections and number of papers presented in each follow: fungicides, 11; small grain diseases, 10; viruses, 13; vegetable diseases, 10; physiology of pathogenic fungi, 9; cereal diseases and pathogens, 10; disease resistance and genetics, 9; forest pathology, 5; factors affecting disease resistance, 6; and 3 in the joint session with the Mycological Society of America.

Conferences included "Plant Disease Survey," "Extension," "Fungicides," "Late Blight of Potato," and a meeting of the Tobacco Disease Council.

The Phytopathologists' dinner, held in the Missouri room of the Hotel Statler on Thursday evening, March 28, was attended by two hundred and twenty.

Council for 1946:

- J. H. CRAIGIE, President (1 yr.), Central Experimental Farm, Ottawa, Canada.
A. J. RIKER, Vice-President (1 yr.), Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.
E. M. JOHNSON, Secretary (3-yr. term expires 1947), Kentucky Agricultural Experiment Station, Lexington 29, Kentucky.
R. M. CALDWELL, Treasurer, and Business Manager of Phytopathology (3-yr. term expires 1946), Purdue University, Lafayette, Indiana.
HELEN HART, Editor-in-Chief, Phytopathology (3-yr. term expires 1948), University Farm, St. Paul 1, Minnesota.
C. H. ARNDT, Agricultural Experiment Station, Clemson College, Clemson, South Carolina.
R. W. GOSS, College of Agriculture, Lincoln 1, Nebraska.
R. J. HASKELL, Extension Service, U. S. Department of Agriculture, Washington 25, D. C.
H. B. HUMPHREY, Box 14, Cosmos Club, Washington 5, D. C.
L. D. LEACH, University Farm, Davis, California.
M. C. RICHARDS, Botany Department, University of New Hampshire, Durham, New Hampshire.
C. M. TUCKER, Botany Department, University of Missouri, Columbia, Missouri.

Representatives:

- A.A.A.S. Council.* J. G. HORSFALL, J. C. WALKER.
Division of Biology and Agriculture, National Research Council. J. C. WALKER.
Board of Editors, American Journal of Botany. A. A. DUNLAP.

Standing Committees:

- Donations and Legacies.* H. E. NEWHALL, R. E. VAUGHN, G. F. WEBER, S. N. ZELLER, N. J. GIDDINGS, Chm.
Extension. O. C. BOYD, O. D. BURKE, H. R. GARRISS, R. J. HASKELL, C. E. SCOTT, C. C. ALLISON, Chm.
Investments. MARVIN E. FOWLER, L. M. HUTCHINS, NEIL E. STEVENS, R. M. CALDWELL, Chm.
Necrology. W. J. ZAUMAYER, H. D. BAEKER, Chm.
Phytopathological Classics. L. C. KNORR, Editor. JOHN NIEDERHAUSER, Business Manager.
Placement. S. J. P. CHILTON, M. W. GARDNER, R. J. HASKELL, L. M. MASSEY, R. S. KIRBY, Chm.
Public Relations. O. D. BURKE, C. J. FIDE, F. J. GREANEY, L. S. HITCHNER, J. H. JENSEN, C. L. LEFEBVRE, E. W. LYLE, J. DUAIN MOORE, A. G. NEWHALL, HARRY R. O'BRIEN, P. P. PIKONE, A. J. RIKER, JOSÉ RODRIGUEZ, R. U. SWINGLE, H. W. THURSTON, J. C. WALKER, K. STARR CHESTER, Chm.
Regulatory Work and Foreign Plant Diseases. C. R. ORTON, R. P. WHITE, E. C. STAKMAN, Chm.
Society Organization. GEORGE ARMSTRONG, O. C. BOYD, F. J. GREANEY, J. B. KENDRICK, J. C. WALKER, FREEMAN WEISS, Chm.

Union of American Biological Societies (and Biological Abstracts). DONALD FOLSOM, L. M. MASSEY, W. C. SNYDER, W. G. STOVER, G. C. KENT, Chm.; HELEN HART and E. M. JOHNSON (ex officio).

Special Committees:

Coordination in Cereal and Vegetable Seed Treatment Research. C. H. ARNDT, F. J. GREANEY, C. M. HEANSELER, K. W. KREITLOW, L. D. LEACH, R. W. LEUCKEL, GEORGE SEMENIUK, M. B. MOORE, Chm.

Fungus Nomenclature. C. M. TUCKER, D. S. WELCH, ERDMAN WEST, G. L. ZUNDEL, J. A. STEVENSON, Chm.

Nomenclature and Classification of Plant Viruses. C. W. BENNETT, L. M. BLACK, JAMES JOHNSON, H. H. MCKINNEY, H. R. McLARTY, FRANK MCWHORTER, FREEMAN WEISS, Chm.

Plant Disease Prevention. J. F. ADAMS, K. D. BUTLER, C. E. F. GUTERMAN, E. C. STAKMAN, DONALD FLETCHER, Chm.

Membership Committee. W. F. BUCHHOLTZ, W. C. SNYDER, G. C. KENT, A. A. DUNLAP, H. A. RODENHISER, E. M. JOHNSON, R. W. SAMSON, Chm.

Publication Problems. M. W. GARDNER, FRANCIS O. HOLMES, A. J. RIKER, Chm.; R. M. CALDWELL and HELEN HART (ex officio).

Reorganization of International Cooperation. H. P. BARSS, G. H. COONS, J. G. HARRAR, OTTO REINKING, J. A. STEVENSON, E. C. STAKMAN, Chm.

Standardization of Fungicidal Tests. M. C. GOLDSWORTHY, C. S. HOLTON, J. G. HORSFALL, M. B. MOORE, C. F. TAYLOR, H. W. THURSTON, J. D. WILSON, S. E. A. McCALLAN, Chm.

Terminology (Nomenclature) of Immunology and Use of Technical Words. D. L. BAILEY, W. H. BURKHOLDER, DONALD FOLSOM, M. W. GARDNER, Chm.

War Committee. J. G. LEACH, E. C. STAKMAN, I. E. MELHUS, Chm. (Executive Committee).

Coordination of Field Tests with New Fungicidal Dusts and Sprays. J. D. WILSON, Chm. (Committee to be announced).

Temporary Committees for 1945:

Auditing. J. RALPH SHAY, R. W. SAMSON, Chm.

Resolutions. C. T. GREGORY, H. C. MURPHY, C. M. TUCKER, Chm.

Temporary Committees for 1946:

Publication of Special Material. H. P. BARSS, J. G. LEACH, W. H. TISDALE, DONALD CATION, Chm.

Sustaining Contributors. R. J. HASKELL, F. L. HOWARD, J. J. CHRISTENSEN, Chm.

Report of the Secretary. At the time of the last Council meeting on December 11, 1944, in Cincinnati, Ohio, the membership was 1089. On December 31, 1945, the total was 1100, a net gain of 11 members. Included in the 1100 were 38 new members and 46 reinstatements. During the period from December 11, 1944, to December 31, 1945, the Society lost 73 members: 9 by resignation, 9 by death, and 55 suspended for non-payment of dues.

Ninety-three names were presented to the Society for membership, 39 of whom applied in 1945 and 54 in 1946; thus the total membership after the election on March 30 was 1193. The 39 applying in 1945 will be placed in the 1945 report, while the 54 applying in 1946 will be included in the totals for that year.

Report of the Treasurer. Statement of accounts for the year ending September 30, 1945.

Receipts:

Balance from 1944			\$2016.34
Annual dues:			
1944		\$ 58.00	
1945		4731.33	
1946		104.00	\$4893.33
30-Year Index			5.50
Sales			42.41
Sustaining contributors			200.00
Unclassified receipt			114.25
Total receipts			<hr/> 3255.49
			\$7271.83

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY:

1944	\$ 46.40	
1945	3772.35	
1946	82.20	\$3900.95

Transferred to PHYTOPATHOLOGY for:

Sales:

Phytopathology	43.41	
30-Year Index	5.50	48.91
Secretarial work and expenses, Office of Secretary		218.28
do, Office of President		56.08
do, Office of Treasurer		275.75
Printing and stamped envelopes		295.46
Stamps		6.00
Money returned for overpayment of dues		16.00
Bank charge		1.95
Exchange charge		.85
Donation to Union of American Biological Societies		50.00
Annual meeting expenses		23.53

Total expenditures	\$4893.76
Balance on hand Sept. 30, 1945	2378.07

\$7271.83

Sinking Fund. Arlington and Fairfax Building and Loan Certificate No. R 1423 for 10 shares (value \$1000) transferred to Arlington and Fairfax Building and Loan Association Savings Share, Account Certificate No. 70 (value \$1000) January 1, 1945. There was no change in the principle amount of the sinking fund during the past year, the total remaining \$9676.00. It is invested as follows:

First mortgage note, at 4½ per cent interest, deposited with McLaehlen Banking Corporation for collection	\$ 500.00
U. S. Savings Bond, Series G, 2½ per cent	1000.00
Invested with the following:	
Columbia Permanent Building Association (accrued dividends \$52.10)	552.10
District Building and Loan Association (accrued dividends \$152.29)	1652.29
National Permanent Building Association (accrued dividends \$263.30)	2263.30
Northwestern Federal Savings and Loan Association	2000.00
Perpetual Building Association (accrued dividends \$104.20)	1104.20
Prudential Building Association (accrued dividends \$26.41)	202.41
Arlington and Fairfax Building and Loan (accrued dividends \$15.00)	1015.00

\$10289.30

Less interest due PHYTOPATHOLOGY	613.30
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\$ 9676.00

The Lyman Memorial Fund, obtained from voluntary contributions, now totals \$3195.82. The whole amount is invested with the Brookland Building and Loan Association, at 2½ per cent. The account for 1945 is as follows:

Balance on hand, Oct. 1, 1944	\$3384.85
Dividends, Dec. 31, 1944 to June 30, 1945	94.01
Voluntary contributions	20.00
Memoir	.50

\$3499.36

Less interest due PHYTOPATHOLOGY	303.54
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\$3195.82

Additional Endowment:

War Savings Bond, Series F	
Total, Sept. 30, 1944	\$1050.00
Received Oct. 1, 1944 to Sept. 30, 1945	50.00
War Savings Stamps	
Total, Sept. 30, 1945	7.00

\$1107.00

Report of the Business Manager. The total number of nonmember subscribers was 554 on November 30, 1945, representing a net gain of 71 for the year 1945. These consisted of 338 domestic, 32 Canadian, and 184 foreign subscribers. Included in the domestic subscriptions for later foreign shipment are 30 for the Chinese Ministry, 41 for the USSR, and 7 for The Netherlands; also 3 complimentary domestic subscriptions. There were 31 subscription cancellations and suspensions in 1945. Not included in the reported subscriptions for 1945 are 55 subscriptions for the current volume, number 35, by the American Library Association, to be held by the Society for eventual shipment to foreign countries as may be designated by that association.

The sales of back volumes and issues during 1945 totaled \$1409.70. This included, among the larger orders, amounts of \$207.00 from West African Cacao Research Institute, Tafo, Gold Coast, and \$208.30 from the Acme Agency, Buenos Aires, Argentina.

Statement of accounts for the year ending September 30, 1945.

Receipts:

Balance from 1944		\$ 6372.55
Subscriptions		
1944	\$ 36.35	
1945	2924.73	
1946	264.20	
1947	27.50	\$3252.78
Member subscriptions:		
1944	46.40	
1945	3772.35	
1946	82.20	3900.95
Sales of back numbers of PHYTOPATHOLOGY		1409.70
Sale of Membership List		1.00
Advertising		
1944	327.61	
1945	807.18	
Membership List	275.00	1409.79
30-Year Index		105.00
Interest on Sinking Fund		
First mortgage	21.50	
Building and Loan	80.00	
U. S. Bond Series G	25.00	126.50
Interest on current funds		145.08
Grant from Rockefeller Institute		600.00
Allowance on reprints		367.17
From authors for excess illustrations		160.44
Total receipts		11478.41
		<hr/>
		\$17850.96

Expenditures:

Printing, distributing, and storing PHYTOPATHOLOGY:			
Vol. 34, no. 9	\$708.93		
10	680.40		
11	532.24		
12	733.09		
Vol. 34, Index	203.94		
Vol. 35, no. 1	745.42		
2	493.24		
3	581.06		
4	507.50		
5	731.06		
6	922.62		
7	716.65		
8	665.37	\$8221.52	
Postage, PHYTOPATHOLOGY	404.17	\$8625.69	
Secretarial work and office expense, Editor-in-Chief		741.62	
do, Advertising Manager		101.73	
Postage for Advertising Manager		5.49	
Commission for Advertising Manager (1944)		150.00	
Expenses of Advertising Manager		19.12	
Secretarial work for Business Manager		314.25	
Stamps		7.00	
Printing and stamped envelopes		184.62	

Office supplies	19.75	
Miscellaneous	33.60	
Postage, 30-Year Index	8.36	
Refund, subscription and sales	31.20	
Bank charges	1.60	
Printing Membership List	276.02	
Checks returned by bank	12.00	
		<hr/>
Total expenditures		\$10532.05
Balance on hand:		
Checking account	2374.14	
Northwestern Federal Savings and Loan	4944.77	7318.91
		<hr/>
		\$17850.96

The 30-Year Index. Summary of receipts and expenditures October 1, 1944 to September 30, 1945:

Balance in excess of expenditures, Sept. 30, 1944	\$118.48
Receipts Oct. 1, 1944, to Sept. 30, 1945	105.00
	<hr/>
Expenses Oct. 1, 1944, to Sept. 30, 1945	\$223.48
	8.36
	<hr/>
Balance in excess of expenditures, Sept. 30, 1945	\$215.12

Membership List Account, 1944-1945.

Receipts for advertising		\$324.98
Printing, 1500 copies	\$268.02	
Postage for mailing	8.00	276.02
		<hr/>
		\$ 48.96
Membership lists sold		1.00
		<hr/>
Balance on hand, Sept. 30, 1945		\$ 49.96

Report of the Auditing Committee, as of September 30, 1945. We have examined the books of the Treasurer of the American Phytopathological Society and of the Business Manager of Phytopathology for the period October 1, 1944, to September 30, 1945, and find all funds, receipts, and expenditures of the Society and of PHYTOPATHOLOGY properly and clearly recorded and accounted for. Much credit is due Miss Melba K. House for the excellent state of the books.

Signed: R. W. SAMSON, *Chairman*
J. RALPH SHAY

Report of the Advertising Manager. PHYTOPATHOLOGY carried a total of 95 paid subscriptions during the year 1945. There were 52 full page, 32 half-page, and 10 quarter-page advertisements.

The gross income from advertising was \$1255.00. The net income to the Society will appear in the Treasurer's report. Advertising agencies get a 15 per cent commission, and a 2 per cent discount is allowed when bills are paid within 10 days. This accounts for the difference between the gross and the net income.

In addition to paid advertisements, the Journal has carried 1 full-page, 9 half-page, and 10 quarter page announcements for the Society in the advertising section.

The present advertising manager wishes to express his appreciation of the fine help and cooperation of the previous advertising manager, S. L. Hopperstead.

Report of the Editor-in-Chief. Two hundred and two individuals utilized the 1032 pages of volume 35 of PHYTOPATHOLOGY to publish 100 articles, 34 notes, 39 abstracts, and 7 biographies. The volume contained 216 text figures and 7 portraits; and tabular material constituted 11.5 per cent of the 975 pages utilized for scientific papers. Three reports and three announcements were published for the Society in the volume.

On March 1 1946, there were 64 papers on hand: 22 have been accepted for publication, 17 are being revised by the authors, and 25 are under consideration by the editorial board. Between January 1, 1945, and March 1, 1946, seven papers have been withdrawn or rejected.

A manual on "Preparation of manuscripts for Phytopathology" has been written by Dr. A. J. Riker, a member of the editorial board. The American Phytopathological

Society authorized the manual at its meeting in Cincinnati, Ohio, in December, 1944. Publication is planned for 1946.

The editor is pleased to acknowledge the financial assistance given PHYTO-PATHOLOGY in 1944 and 1945 by the Rockefeller Institute for Medical Research.

Every member of the Editorial Board has cooperated in reviewing manuscripts submitted for publication: the work of the editorial office would have been impossible without their assistance. It is also a pleasure to acknowledge the advice and help of the Science Press Printing Company in meeting the difficulties besetting scientific publications during war times.

Report of Society Representative on the National Research Council for 1945.

Activity of the Division of Biology and Agriculture during the year may be summarized with respect to matters related to our field as follows:

1. The Division has been active in keeping representatives informed as to the progress of the bills before Congress concerned with a National Research Foundation and bringing its influence to bear on the need of proper emphasis upon research in biology in the formulation of this legislation. At your president's request a statement was prepared by your representative which was filed with the appropriate Committee of Congress.

2. A Microbiological Board within the Division was recommended to function as a central clearing and guidance agency in this field. Particular interest at the moment is concerned with antibiotics which obviously reaches into the field of plant disease control. While such a board has not as yet been set up, preliminary conferences have been held to consider organization and the possibilities of financial support for such organization.

3. The Office of Scientific Personnel continues to function with financial support by the American Institute of Physics, the American Mathematical Society and the Mathematical Association of America, the Geological Society of America, and the newly formed American Psychological Association. During the war, Biology was represented financially by a special grant from the National Academy (\$3000). At present biological societies are not contributing to this central effort.

4. The Committee on the Training of Research Workers in Agriculture is making a careful study of the needs of fellowships in this field. If the Fellowship program included in the National Research Foundation legislation does not materialize, it is expected that efforts will be made by the Division to initiate a strong fellowship program in Agriculture.

Report of the Representative to the Board of Editors, American Journal of Botany.

During 1945 this representative reviewed, at the request of the Editor-in-Chief, four papers dealing with subjects of phytopathological interest submitted for publication in the American Journal of Botany. Three of these papers have already appeared in that journal. In two instances the names of well-qualified reviewers have been suggested to the Editor-in-Chief for papers dealing with plant diseases or plant-disease fungi.

This relationship with the American Journal of Botany has been a pleasant one and it is sincerely hoped that the suggestions for improvement of the papers examined have been of value.

Report of the Necrology Committee. Death date of member not previously reported:

JAMES IRA P. MCMURPHY, November 2, 1943

Deaths of members during 1945:

J. P. JOLIVETTE, February 2
A. E. EDGEcombe, March 30
L. R. JONES, April 1
M. B. WAITE, June 5
L. H. LEONIAN, June 7
C. J. KING, October 17
R. B. HARVEY, November 4

Report of the Manager of Phytopathological Classics. Report for the fiscal year beginning October 1, 1944, and ending September 30, 1945:

Classic No. 1:	On hand, Oct. 1, 1944	15	
	Sold during year	9	\$ 4.50
	On hand, Sept. 30, 1945	6	
Classic No. 2:	On hand, Oct. 1, 1944	216	
	Sold during year	8	4.00
	On hand, Sept. 30, 1945	208	
Classic No. 3:	On hand, Oct. 1, 1944	306	
	Sold during year	8	4.00
	On hand, Sept. 30, 1945	298	

Classic No. 4: On hand, Oct. 1, 1944	366	
Sold during year	7	5.25
On hand, Sept. 30, 1945	359	
Classic No. 5: On hand, Oct. 1, 1944	604	
Sold during year	10	12.50
On hand, Sept. 30, 1945	594	
Classic No. 6: On hand, Oct. 1, 1944	692	
Sold during year	11	8.25
On hand, Sept. 30, 1945	681	
Classic No. 7: On hand, Oct. 1, 1944	716	
Sold during year	14	10.50
On hand, Sept. 30, 1945	702	
		<hr/>
		\$49.00
Value of books sent out (fiscal year 1944 ⁸ -1945)		\$49.00
Overpayment (retained as credit)		.25
Money received on order of previous year		.75
		<hr/>
		\$50.00
Money received during fiscal year 1944-1945		\$44.50
Gratis set of Classics for Editorial use (L. C. Knorr)		5.00
Due on account		.50
		<hr/>
		\$50.00

Assets:

Cash balance on hand, Oct. 1, 1944	\$463.03
Receipts during year	44.50

Total	\$507.53
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Liabilities:

Error in financial records inherited from previous years	10.00
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Balance on hand, Sept. 30, 1944	\$497.53
Total due on account, Sept. 30, 1945	\$ 20.25

Your attention is called to the fact that there remained only 6 copies of Phytopathological Classic No. 1 on September 30, 1945, and this classic is now out of print. Since most orders received are for complete sets, it behooves us to consider the advisability of reprinting this Classic No. 1. A second edition of 500 copies would probably meet our needs for several decades, and would allow our future customers to buy and maintain a complete set of Phytopathological Classics.

Report of Placement Committee. The Placement Committee has had the busiest year since the employment agency was established. This came about as a result of an unprecedented demand for plant pathologists and the return of veterans from the armed forces.

During 1945, fifty-two plant pathologists had applications filed with the committee. One hundred and fifty-five applications were sent to prospective employers who referred thirty-nine positions to the committee.

Incomplete records show that at least six plant pathologists obtained positions through the efforts of the Placement Committee.

Report of the Public Relations Committee for 1945. The reconstituted Committee has adopted a new means for acquainting the lay public with phytopathological progress by issuing its "IDEAS for Stories Featuring the Conquest of Plant Disease through Modern Research." Each "IDEA" is a brief synopsis that may be developed into a feature article. "IDEAS" are sent, a few at a time, to editors of 90 leading national magazines. If an editor is interested in the development of an "IDEA," the matter is referred to a specialist in that phase of phytopathology, who prepares the article to meet the editor's specifications. The editor is obligated to permit the scientist who provides the material to check the completed article for scientific accuracy, and to reimburse the scientist at his customary rate.

During the year, 31 "IDEAS" were issued. More than half of these were selected as bases for feature stories by editors of *Successful Farming*, *Fortune*, *Scientific*

Monthly, Southern Agriculturist, Food Packer, and other magazines. The remaining "IDEAS," those not preempted by national magazines, were distributed to a key man in each State for issuance to newspapers and radio stations. Many congratulatory letters testify to the extensive use of these releases. The Committee is now represented on the editorial staff of the Scientific Monthly.

The work of the Committee has been self-supporting. The committee members were requested to contribute to the work of the Committee 10 per cent of amounts received for published articles, and the proceeds have been somewhat more than enough to pay the out-of-pocket expense of the Committee—stationery and postage.

The Committee takes this opportunity of thanking those members of the Society who have cooperated in the public relations work during 1945, either by assisting the Committee directly or through their free-lance efforts to the same end. For the future, "IDEAS" submitted by any member of the Society will be most welcome and will materially aid the work of the Committee.

Report of the Committee on Biological Abstracts and the Union of Biological Societies. *Two Decades.*—Twenty years ago a Joint Publications Committee, representing the National Academy of Sciences, Union of American Biological Societies, and American Association for the Advancement of Science, laid firm the foundations of Biological Abstracts. Nineteen volumes, comprising abstracts of some 425,000 contributions to research, have now been published—a significant accomplishment in itself. More important, however, is the fact that an agency for abstracting the entire world's output in biology was set going and has now been put on a secure footing for steady and rapid expansion in the years to come. This agency has also taken the initiative in establishing a world-wide system of cooperation among biologists. With more than 3,000 collaborators active in preparing and editing its material, Biological Abstracts has become one of the largest cooperative enterprises ever instituted by a scientific group. Plans for sectional publication have also been developed which represent a successful enterprise between institutional and specialized individual needs. Last year a new section—Abstracts of Human Biology—was added to the seven already in operation.

During the 1926-35 period, organization of the enterprise was made possible by large foundation subsidies. The difficulties of the succeeding transition period are now so well known among biologists as to require no summary here; suffice it to say that Biological Abstracts has weathered the storm, it stands on its own feet, its revenues have been built up to a point where continuance on a sound financial basis seems fully assured. Annual budgets have increased from \$40,000 in 1938 to \$89,000 in 1945. This reflects a steady healthy growth based in the main on increases in earned income—a growth controlled through appreciation of the service by the scientists themselves as reflected in their increasing subscriptions and support in other ways.

One Year. In the full edition of volume 19 (1945), 23,446 abstracts were published. At the beginning of 1945 the regular coverage included 1,900 periodicals. During the year it had increased to some 2,130, many of the later additions being European journals which had been unavailable through the war years. In 1945 some 444 new subscribers were added. An operational surplus of about \$5,000 was reported, made possible by contributions including \$13,655 from the industries, continuance of which cannot be counted upon. The cost of producing Biological Abstracts has more than doubled within the past four or five years. Salaries have had to be increased, and every phase of operation is costing more. A slight increase for subscriptions to the complete edition was thus made necessary, but the price of Section D (Phytopathology, Plant Physiology, Plant Anatomy, Paleobotany, Systematic Botany, Agronomy, Horticulture, Forestry, Pharmacognosy, Pharmaceutical Botany, and Ecology) remains the same—only \$6.00 (foreign \$6.50).

Plant Sciences. In Section D, Abstracts of Plant Sciences, approximately 5,500 abstracts were published during 1945; 883 of these were in the subsection on plant pathology, and 275 further abstracts containing at least incidental contributions on plant diseases appeared in other sections, making a grand total of 1,158. Subscribers to Section D increased by 96 during the year; of these some 25 were in response to the letter sent by this Committee to members of the American Phytopathological Society. It might be added that about 30 new subscribers resulted from a similar letter sent out to members of the Botanical Society of America by its Secretary.

Some time ago this Committee endeavored to establish a subsection for reviews of films dealing with plant diseases and pests. Much interest in the idea was shown in correspondence with research people and college and extension teachers. No one during the war, however, seemed to have the necessary time to implement the proposal. A comparable subsection established by the bacteriological groups has already proved its worth in their several fields of interest. The Editor of Biological Abstracts would like to see a section established for reviewing films of interest to the whole biological field. It is believed that teachers, extension workers, and researchers would benefit from such a

section. The Committee therefore recommends that the Society accept the responsibility of reviewing for Biological Abstracts films relating to plant pathology, perhaps through its Extension Committee.

The Future. It is hardly too much to say that the greatest impediment to the advancement of science is the lack of fully effective means by which the findings of the scientists themselves, and especially those of other nations, can be mobilized, brought to light, and put to work. Progress in carrying out the postwar plans for a more adequate and worldwide coverage will depend largely on how far the users of Biological Abstracts are willing to go in concrete acknowledgment of its value, through a steady increase in the number who subscribe. Biological Abstracts enters its third decade firmly established and willing and eager to give to scientists what they want and are willing to pay for; it is their cooperative enterprise.

Report of the Committee on Resolutions. Be it resolved that The American Phytopathological Society express its grateful appreciation to the following for their contributions to the success of the 37th annual meeting:

W. D. Valleau and Stephen Diachun for their arrangement of the section programs.

Carroll W. Dodge for arranging the musical program for the Society dinner, and his general assistance as the Society's local representative.

J. M. Hutzler, Executive Assistant A.A.A.S., for his helpful cooperation in assigning rooms and providing projection equipment.

The management of the Statler and Jefferson Hotels, particularly Mr. Bush of the Hotel Statler and Mr. Lucy of the Hotel Jefferson, for their assistance and courtesies in making their facilities available.

The newspapers—the St. Louis Post-Dispatch, the St. Louis Globe-Democrat, and the St. Louis Star-Times—for their effective handling of news coverage.

Respectfully submitted,

C. T. GREGORY

H. C. MURPHY

C. M. TUCKER, *Chairman*

Elections and Appointments. A committee from the Council opened and counted the ballots, results of which were announced to the Society at the banquet the evening of March 28: J. H. Craigie, President; A. J. Riker, Vice-President; C. M. Tucker, Councilor-at-large.

The Council recommended and the Society approved the appointment of Helen Hart as Editor-in-Chief for a three-year term, 1946 through 1948; W. H. Burkholder, Editor for a three-year term, 1946 through 1948; Donald E. Bliss, H. Loran Blood, A. L. Harrison, and Ray R. Hirt, Associate Editors for a three-year term, 1946 through 1948; Paul E. Tilford, Advertising Manager of Phytopathology for a one-year term; L. C. Knorr, Editor for Phytopathological Classics for a one-year term; John Niederhauser, Business Manager of Phytopathological Classics for a one-year term.

Representatives of the Society, new committees, and changes in committee personnel are given on the previous pages of this report.

Ninety-three applicants were elected to membership in The American Phytopathological Society.

Reports of Officers, Representatives, and Standing Committees are published on the previous pages. According to action of the Society at the Philadelphia meeting, reports of Special and Temporary Committees are not to be published in the annual report. All committee reports submitted were considered by the Council. The reports recommended for approval by the Council were accepted by the Society.

The Society approved the following recommendations by the Council.

1. That the paper entitled "Preparation of Manuscripts for Phytopathology," mentioned in the report of the Committee on Publication Problems and prepared in accord with the instructions of the Society at Cincinnati, be published in PHYTOPATHOLOGY and that the manuscript be held in type four months in order to give those who wish copies an opportunity to purchase them from Science Press Printing Company, Lancaster, Pennsylvania.

2. That the announcements of Tentative and Recommended Methods of the Committee on Standardization of Fungicidal Tests be published in Phytopathology as advertisements, whenever space is available, without cost to the Committee.

3. The appointment of J. G. Horsfall as the Society's representative on the A.A.A.S. Council for a two-year term.

4. The appointment of J. C. Walker as the Society's representative on the Division of Biology and Agriculture, National Research Council, from July 1, 1946 through 1948.

5. The appointment of A. J. Riker on the Committee on Public Relations.

6. The appointment of H. R. McLarty on the Committee on Nomenclature and

Classification of Plant Viruses in place of H. Earl Thomas, who was appointed last year but refused to serve.

7. That the Secretary write Mrs. L. R. Jones a letter, on behalf of the Society, expressing the Society's appreciation of the bequest of \$100 to the Society, left by the late L. R. Jones.

8. The establishment of a Special Committee on Coordination of Field Tests with New Fungicidal Dusts and Sprays, and that J. D. Wilson be appointed chairman with the power to name his committee.

9. That the Tobacco Disease Council be asked to study the possibilities of eradication of blue mold of tobacco and make recommendations to the Council of The American Phytopathological Society.

10. That it be the sentiment of The American Phytopathological Society that a means of affiliation of Phytopathological Societies in other countries be provided for in the revised Constitution, provided such societies wish to affiliate. That each affiliate be given the minimum of privilege of representation on the Council. That the proceedings of such affiliates be published in Phytopathology at the Society's expense.

11. That the name of the New England Division be changed to the North Eastern Division, as set forth in a petition presented to the Council.

12. That the 38th annual meeting be held Saturday, December 28, through Monday, December 30, 1946, in Cincinnati, Ohio, in Cleveland, Ohio, or in Chicago, Illinois.

ANNOUNCEMENT

The thirty-eighth meeting of the American Phytopathological Society will be held at the Netherland Plaza Hotel, Cincinnati, Ohio, December 28-30, 1946.

JAMES PETER JOLIVETTEJuly 20, 1915–February 2, 1945

James Peter Jolivette was graduated from the University of Wisconsin in 1937 with the degree of Bachelor of Science in Agriculture, and in 1941 he received the degree of Doctor of Philosophy from the same institution.

From 1941 to March 20, 1942, he was Instructor in Plant Pathology, University of Wisconsin, in charge of the truck crop disease field laboratory at Kenosha, Wisconsin. During his college days, he was in the Reserve Officers' Training Corps. When he graduated in 1937, he was commissioned as Second Lieutenant, and in 1941, First Lieutenant, in the Officers' Reserve Corps, U. S. Army. He was called into active military service March 20, 1942. From May, 1943, until his untimely death on Luzon, P. I., by enemy fire, he was Captain of his Company, Co. L, 20th Infantry, 6th Division, U. S. Army.

As an investigator in his chosen field of plant pathology, Dr. Jolivette showed marked ability and originality, both in fundamental research and in its practical applications. As a soldier, his courage and leadership were an inspiration to his men. The memory of him as a soldier, as a scientist, and as a man will continue to be a lasting inspiration, especially to those who were fortunate enough to know him.

ALBERT EDWARD EDGECOMBEFebruary 5, 1897–March 30, 1945

Albert Edward Edgecombe was graduated from Queens University (Ontario) in 1923 with the degree of Bachelor of Arts, and in 1929 he received the degree of Doctor of Philosophy from the University of Chicago.

From 1929 to 1939 he served as Assistant Professor of Botany and as Associate Professor from 1939 till his death, all at Northwestern University. His special field was mycology, and his researches dealt with certain rusts and dermatophytic fungi. His teaching activities covered a wide variety of botanical subjects.

Dr. Edgecombe was a charter member of the Mycological Society of America and a member of several other scientific organizations. He was a man of high standards, both in research and in teaching, of indefatigable industry, and of broad interests. He believed that assiduity should be a chief criterion of one's success.

LEWIS RALPH JONES

December 5, 1864–April 1, 1945

Born and reared on a Wisconsin farm, Lewis Ralph Jones later attended Ripon College and the University of Michigan (Ph.B., 1889), taught "natural science" at Mount Morris Academy, Illinois, and in 1889 became Instructor in Natural History at the University of Vermont. Here he was rapidly advanced to assistant and associate professorships, becoming full Professor of Botany in 1893. He was appointed Botanist of the Agricultural Experiment Station in 1889, and continued in these two capacities until he left Vermont in 1910 to found a department of plant pathology in his home State university. Organized for research and training of men and women to carry on the torch of discovery, his new department rapidly became a mecca for graduate students. Professor Jones retired from active service in 1935, but continued his important responsibilities in the National Academy of Sciences and as committeeman, trustee, and counselor.

Of degrees and honors Dr. Jones had many—the much coveted honorary doctorate from Cambridge University (Sc.D., 1930), and others from the universities of Vermont (Sc.D., 1910), Wisconsin (Sc.D., 1936), and Michigan (Ph.D., 1904; LL.D., 1935). He held world-wide memberships in over twenty scientific organizations—with high offices in ten or more. He was one of the chief organizers of the Vermont Botanical Club, Forestry Association of Vermont, Boyce Thompson Institute, Tropical Plant Research Foundation, and American Phytopathological Society and a leading spirit in the Botanical Society of America. In his chosen field of plant pathology, he was the prime mover in the national society, its guardian and guide throughout the formative early years, its first president (1909), and the first editor-in-chief of its official journal *PHYTOPATHOLOGY* (1911–14).

Jones was pre-eminent as a researcher. Among the more outstanding of his contributions are those on Bordeaux mixture, potato diseases, sap flow in sugar maple, bacterial soft rot of vegetables, the nature and control of diseases in plants, the development of resistant varieties, and the effects of environment on infection and pathogenesis.

Professor Jones held the rare distinction of standing high not only as researcher, but also as practical agriculturist, organizer, administrator, and teacher. It is as a teacher, however, that his influence will perhaps extend furthest and last longest: first because of the man himself, and second because he knew that he could best serve his time by developing men, programs, and institutions—he was above all a human catalyzer of ideas and ideals. To sit under his tutelage was a liberal education in itself; he taught science "with a difference."

MERTON BENWAY WAITE

January 23, 1865—June 5, 1945

Merton Benway Waite was graduated from the University of Illinois in 1887 with the degree of Bachelor of Science, and in 1919 the University of Maryland conferred on him the degree of Doctor of Agriculture.

From 1887 to 1888, Dr. Waite served as assistant to Dr. T. J. Burrill at the University of Illinois. From 1888 to the time of his retirement in 1935, Dr. Waite was employed in the U. S. Department of Agriculture, at first with Dr. B. T. Galloway in the old Section of Vegetable Pathology and later as head of the Office or Division of Fruit Disease Investigations, in the Bureau of Plant Industry. From 1930 to 1938, he was a lecturer on plant diseases and from 1932 to 1938, also on plant ecology, in the Graduate School of the Department of Agriculture.

Dr. Waite was a charter member of The American Phytopathological Society and a member of a number of other scientific organizations. He was an unusually keen observer, an enthusiastic, inspiring leader, with a wealth of knowledge, clear vision, and well-balanced judgment. His scientific attainments are recognized the world over and are a continuing memorial to him. His genial smile and cordial greeting will be greatly missed by his many friends.

LEON HATCHIG LEONIAN

February 27, 1888—June 7, 1945

Leon Hatchig Leonian was graduated from the University of Kentucky in 1916 with the degree of Bachelor of Science and, from the University of Michigan, he received the degree of Master of Science in 1917 and the degree of Doctor of Philosophy in 1922.

Dr. Leonian served successively as Assistant Research Horticulturist at Clemson College Agricultural Experiment Station, 1917-18; Assistant Professor of Botany and Plant Pathology at New Mexico State College and Experiment Station 1918-1922 (part time); and in the College of Agriculture and Agricultural Experiment Station of West Virginia University, as Assistant Professor of Plant Pathology from 1922 to 1926; Associate Professor of Plant Pathology, 1927-1936; and Professor of Mycology and Mycologist, from 1936 to the time of his death.

As a researcher, Dr. Leonian did notable work, particularly on the physiology of fungi, and as a teacher, he was a favorite with his students. His frank ways and pleasant smile will be greatly missed by his many friends and associates.

CHALMERS JACKSON KING

October 26, 1893–October 17, 1945

Chalmers Jackson King was graduated from Clemson College in 1913 with the degree of Bachelor of Science, and pursued graduate work there in 1914.

From 1914 to 1916 he was Instructor in Soils at Clemson College and Assistant Chemist in the South Carolina Agricultural Experiment Station. He went to Arizona in 1917 as Assistant in Biophysical Investigations with the Bureau of Plant Industry, U. S. Department of Agriculture. In this position through 1920 he conducted research in cotton and citrus nutrition and water requirements. From 1921 until his death he was Superintendent of the U. S. Cotton Field Station at Sacaton, Arizona. Under his direction Sacaton became an important center for cotton research. From 1935 on, he was in charge of the Federal program of cotton research in the Southwestern States.

“C. J.”, as he was familiarly known to his associates and to cotton producers throughout Arizona, California, New Mexico, and west Texas, devised practical control measures for the crazy-top disorder of cotton, for *Phymatotrichum* root rot, and for other diseases under irrigated conditions. He contributed much to his associates in inspiring interest in cotton research and to cotton producers in helping them to understand and to overcome practical production problems.

RODNEY BEECHER HARVEY

May 26, 1890–November 4, 1945

Rodney Beecher Harvey was a graduate in the pharmacy course at Purdue University in 1912 and was graduated from the University of Michigan in 1915 with the degree of Bachelor of Science. In 1918, he received the degree of Doctor of Philosophy from the University of Chicago and the honorary degree of Doctor of Science from Purdue University in 1939.

Dr. Harvey served as Assistant Botanist, Eli Lilly and Company, 1912–13; Assistant Botanist, University of Michigan, 1915; Microanalyst, 1915–1919, and Physiologist, 1919–1920, Bureau of Plant Industry, U. S. Department of Agriculture; and at the University of Minnesota and Agricultural Experiment Station he served as Assistant Professor of Plant Physiology, 1920–1923; Associate Professor of Plant Physiology, 1923–1929; Associate Professor of Plant Physiology, Agricultural Botany, and Horticulture, 1929–1930; and Professor of Plant Physiology from 1931 to the time of his death, except certain periods while on temporary leave. During 1936–37, he served as Director of the Citrus Laboratory, Dunedin, Florida; and during 1942–43, he was Director, Division of Industrial Microbiology, General Mills.

He was honored by being granted a Guggenheim Memorial Foundation Fellowship, 1927–28. He was a charter Member of the American Society of Plant Physiologists (Secretary, 1923–25; Vice-president, 1931; President, 1936; and Executive Committee, 1937–). He was a member also of the Botanical Society of America, Ecological Society, and the American Phytopathological Society.

Dr. Harvey did notable work on a wide range of problems in the field of plant physiology. Some of these that have close relation to plant pathology are: absorption of electrolytes by plants; enzymes of respiration; reactions of protoplasm to low temperatures; ethylene ripening of fruit; and the nature of toxic action. He was a progressive thinker and was unusually active in whatever he undertook.

ASSOCIATION OF *XANTHOMONAS PHASEOLI* AND THE COMMON BEAN-MOSAIC VIRUS, MARMOR *PHASEOLI*. II. DISSOCIATION STUDIES OF *X. PHASEOLI*

FLORENCE HEDGES¹

(Accepted for publication March 10, 1946)

INTRODUCTION

In earlier studies (4, 5) the writer found that following a long-continued uninterrupted *in vivo* association within stringless Green Refugee variety of common bean (*Phaseolus vulgaris* L.) of the seed-borne infective agents *Xanthomonas phaseoli* (E. F. Sm.) Dowson and bean virus 1 (*Marmor phaseoli* Holmes), the severity of the virus symptoms had greatly increased. Whether this was due wholly or in part to the association of bean virus 1 with the bacterium, to the chance entrance of another virus into the host (5, p. 689), or to the production of a mutant of bean virus 1 was not known. The fact remained that, beginning with the 38th serial passage from bean plant to bean plant of the juice containing the virus and the bacterium, an ultra-severe form of mosaic was produced in 100 per cent of the plants (5, Fig. 1). This persisted from the 38th through the 50th serial passage.

The bacterium, on the contrary, had diminished in its virulence to the point of complete failure to produce symptoms of bacterial infection and to maintain itself in its "typical" virulent S² yellow form in the host tissues. This condition persisted for 10 serial passages after which bacterial symptoms re-appeared on the inoculated primary leaves and it was again possible to isolate typical *Xanthomonas phaseoli* from the inoculated bean plants. The 65 checks in this serial passage had no sign of seed infection.

In the interim mildly pathogenic S opaque-white colonies and nonpathogenic S pink ones appeared in the poured plates from the inoculated plants. These aberrant types that had previously been observed from time to time in association with "typical" S yellow colonies were believed to be variants of *Xanthomonas phaseoli*.

In the present communication the writer reports studies, by means of dissociation experiments, of the changes that the bacterium had undergone during its association with the virus *in vivo*.

MATERIALS AND METHODS

The cultures selected for this study were as follows:

1. A virulent S yellow isolate of *Xanthomonas phaseoli* from the 34th

¹ Formerly associate pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, Plant Industry Station, Beltsville, Md.

² Abbreviations are used to designate the type of colony: S, smooth and shining; \pm S, more or less smooth and shining; R, rough; \pm R, more or less rough; RS, intermediate between rough and smooth or containing both characters (applied to a given culture at a given time); R-S, sometimes rough and sometimes smooth (applied to the white variant of *Xanthomonas phaseoli*).

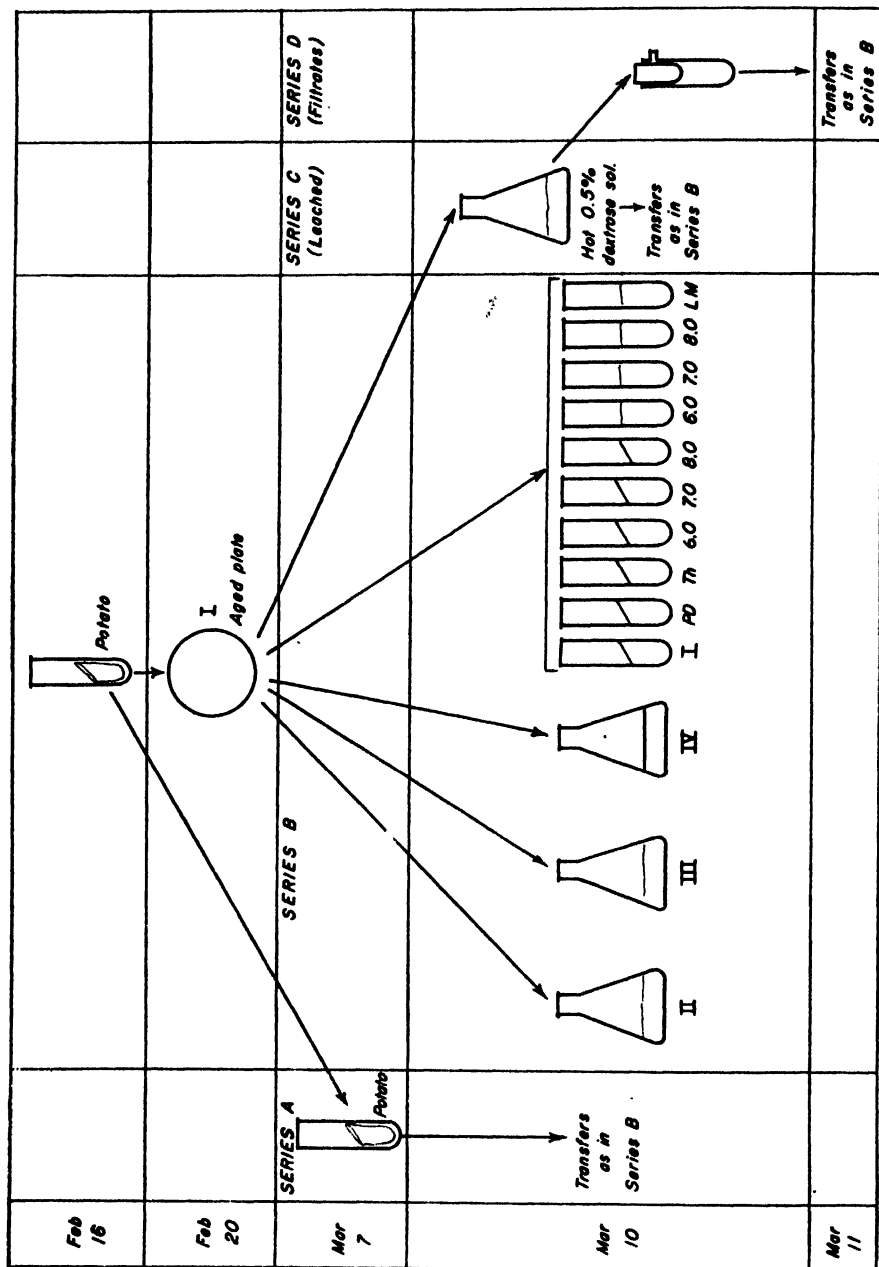


FIG. 1. Diagram of Quirk's dissociation technique used with P(M+P)²¹ and "normal" *Xanthomonas phaseoli*. A. Series from young culture as check. B. Series directly from aged, medium-I plate. C. Series from leached contents of aged plate of medium I. D. Series from filtrates of leached contents of aged plate of medium I (filtration apparatus in refrigerator overnight).

Abbreviations: I-IV, Quirk's potato media; PD, potato-dextrose agar; Th, Thaxter's potato-dextrose agar; 6.0, 7.0, 8.0, pH 6.0, pH 7.0, pH 8.0 beef-infusion agars or broths (agar indicated by oblique line in tube); L.M., litmus milk.

serial passage of infected juice from bean plant to bean plant, marking the expiration of two years' uninterrupted association of the common bean mosaic virus and the bacterium *in vivo*. This isolate, designated P(M + P)³⁴, originated from a single colony among only 5 colonies in the isolation plate; and all 5 colonies of the plate resembled those characteristic of the virulent S yellow type-form of *X. phaseoli*. In the designation P(M + P)³⁴, the P denotes *Xanthomonas phaseoli*, the M denotes the mosaic virus, and the superior figure 34 denotes the 34th serial passage of bean juice containing the virus and the bacterium. The 34th serial passage was the second previous to the disappearance of all symptoms of bacterial infection and the fourth preceding the onset of ultra-severe mosaic symptoms.

2. An S yellow, single-colony culture of "normal" *Xanthomonas phaseoli* isolated 8 months before from a lima bean pod and having no history of association with the mosaic virus. This strain likewise was very virulent. It was designated "normal" *Xanthomonas phaseoli*. Like the preceding, it was transferred from a thinly sown plate.

The technique and the media used were those of Quirk (11), to whom the writer is much indebted for suggestions as to their use. The procedure, diagrammatically shown in figure 1, was as follows:

Series A. A check series of each isolate was made from a young culture on steamed potato cylinder.

Direct transfers from 4-day-old steamed-potato-cylinder cultures of the two isolates, P(M + P)³⁴ and "normal," were made on February 20 to Petri dishes (150 × 15 mm.) containing a thick layer (8 mm. cir.) of hardened Quirk's medium I. The two strains were indistinguishable in the parent potato cultures. The plate cultures on Quirk's medium I were aged 3 weeks at room temperature. At the end of this period the growth of P(M + P)³⁴ was very pale yellow (slightly greenish yellow) with a white margin; the "normal" culture was a deeper greenish yellow with white sectors. These aged cultures were used in Series B, C, and D.

Series B. Transfers of the 2 isolates were made directly from the aged plates to flasks filled to a depth of 1 to 1½ inches with Quirk's media II, III, and IV and to tubes of Quirk's medium I, Potato-dextrose agar, Thaxter's potato-dextrose agar, Beef-infusion agar at pH 6.0, pH 7.0, and pH 8.0, Beef-infusion broth at pH 6.0, pH 7.0, and pH 8.0, and Litmus milk. In other words, the tubes comprised a set of the culture media most commonly used for phytopathogenic bacteria plus Quirk's medium I. Later, steamed potato cylinders, upon which "typical" *Xanthomonas phaseoli* grows very characteristically,³ were included in the tube setup. No beef-extract agars were used.

Series C (leached). In this paper the term "leaching" is applied to a soaking or steeping of material from the aged plate cultures. The proce-

³ Copious, smooth, shining, more or less fluid or "sirupy" growth of varying shades of yellow. If potato cylinders half covered with water are used before they have been allowed to dry out at all, the growth usually so fills the liquid that the cultures can be turned upside down with impunity.

ture was as follows: Fifty cc. of 0.5 per cent dextrose solution was autoclaved in 500-cc. flasks and cooled to 60° C. cir. At this point were transferred to the flasks small pieces of the agar substratum from the aged plates after the greater part of the bacterial growth had been removed with a sterile swab and discarded. About $\frac{1}{3}$ of the layer of agar in the Petri dish was used. The dextrose solution was hot enough to soften the agar and expedite the freeing of any filterable forms that might have penetrated it, and at the same time the solution cooled off too quickly to kill *Xanthomonas phaseoli*, the thermal death point of which is about 50° C. with a 10-minute exposure. The flasks were well shaken and allowed to stand about 20 minutes. Transfers from the leach were then made to the same media as in Series B.

Series D. The suspensions of the two isolates in the 0.5 per cent dextrose leaching solution used in Series C were filtered by gravity through a Chamberland-Pasteur L 3 filter immediately after making the Series C transfers. As this step was reached so late in the afternoon that insufficient filtrate was obtained for inoculum by nightfall, the filtration was completed in a refrigerator held at 10° to 12° C. and transfers like those in the preceding series A, B, and C, were made early the next morning.

Quirk's media I-IV. The following directions for making the special potato media used in these investigations were furnished by Miss Quirk.

Medium I, yeast-potato-mannitol-nitrate agar with oxidized potato-extract base, prepared as follows: Scrub potatoes thoroughly, pare, grind in a meat grinder. To 2 parts distilled water add one part potato. Allow to stand in open glass or enamel receptacle in refrigerator overnight. Next morning stir mixture well to distribute starch. Filter through cheesecloth to let juice and some of the starch pass. Put oxidized juice and starch into large flasks and autoclave one hour at 115° C. Let stand overnight in flasks to precipitate the cooked starch. Decant the supernatant liquid and filter through cotton. Do not add water to make up to volume.

To 500 cc. oxidized potato extract, add 100 cc. yeast solution,⁴ 500 cc. distilled water, and 15 g. agar. Cook mixture for one hour in steamer. Filter through cotton, add the following chemicals: 10.0 grams Mannitol, 0.2 g. NaCl, 0.2 g. K₂HPO₄, 0.2 g. MgSO₄, 0.1 g. CaSO₄, 1.0 g. CaCO₃, and 1.0 g. KNO₃.

Stir chemicals well into hot agar (steam 10 or 15 minutes if necessary). Without filtering mixture, distribute medium in small flasks to a depth of 1 to 1½ inches. Autoclave 20 minutes at 115° C.

Medium II, unoxidized-potato agar (4 per cent). Prepared as Thaxter's potato-dextrose agar (2, p. 154) but with an additional 2½ per cent agar. Slice potatoes thin and immediately cover with distilled water to prevent oxidation. To 2 parts of distilled water add 1 part potato. Steam 20 minutes, or let simmer in a water-bath, below 60° C. for 1 hour. Filter

⁴ Stock yeast solution: 100 g. yeast cakes (Fleishman's), 1000 cc. of distilled water. Cook 4 hours in steamer, stir occasionally, allow yeast to settle. Decant supernatant liquid and autoclave 20 minutes at 115° C.

through cheesecloth and make up to original amount with distilled water. Add 2 per cent dextrose and 4 per cent agar. Cook 1 hour. Filter through cotton. Distribute in small flasks. Autoclave 20 minutes at 115° C.

Medium III, oxidized-potato-extract agar (4 per cent). Prepare potato extract and yeast solution as for medium I. Add 2 per cent of dextrose and 4 per cent of agar. Cook 1 hour. Filter through cotton. Distribute in small flasks to a depth of 1 to 1½ inches. Autoclave 20 minutes at 115° C.

Medium IV, potato-starch medium. Precipitated sterile starch jelly obtained while making oxidized potato extract for media I and III. This cooked precipitated starch is washed well under running tap water. The washed starch jelly is then distributed in small flasks to a depth of 1 to 1½ inches. Autoclave 20 minutes at 115° C. (No ingredients added to the starch jelly in medium IV.)

Pathogenicity testing. The pathogenicity of the dissociated cultures was tested by rub-inoculations on the underside of the primary leaves of young Stringless Green Refugee bean seedlings. Both the undiluted bacterial growth and sterile-distilled-water suspensions of the same were used as inoculum. A large number of noninoculated isolated checks was always held.

The line of Stringless Green Refugee bean used in the pathogenicity tests was the same as that used in the serial passages described in the earlier paper (5, p. 666). The seed was collected by W. J. Zaumeyer from healthy plants in his breeding plots at Greeley, Colorado.

CULTURAL STUDIES

I. *Dissociated Cultures Prior to Filtering*

By the Quirk technique of demonstrating dissociation, most striking differences between the $P(M+P)^{34}$ strain and "normal" *Xanthomonas phaseoli* were readily revealed. This showed to be a fact that which the writer had suspected from the behavior of the bacterium in the serial passages, namely that *X. phaseoli* had undergone considerable change during its two years' uninterrupted association with the virus *in vivo*.

Strain $P(M+P)^{34}$. Transfers from the aged plate before and after leaching (Fig. 1, Series B and C, respectively). The bacterial isolate which had been associated continuously for two years with the virus *in vivo* was readily dissociated into:

1. A dominant white form, varying from R to S and only mildly pathogenic.
2. A subordinate S yellow form, the "typical" virulent form.
3. An S pink form.⁵

On all solid media, including those in the tube series (Fig. 2, A and C), inoculated either before or after leaching, the \pm R white variant appeared

⁵ This is believed to be the pink form encountered from time to time, usually as a minority group, in plates from bean plants inoculated with the two associated infective agents. If so, it is nonpathogenic. In the dissociated cultures under discussion, it always appeared in conjunction with either the yellow or white forms.

first and was very much to the fore. Relatively little of the virulent S yellow type developed in transfers made either before or after leaching but its best development was on Quirk's medium IV, inoculated before leaching. There was only a trace of it in the tube transfers, namely on medium I, potato-dextrose agar, and Thaxter's agar made before leaching, and only on the two last mentioned made after leaching.

No S pink form appeared in transfers made prior to leaching with the hot dextrose solution. After leaching, this form reached its greatest development in transfers to flasks of Quirk's media II and III. In the tube series it appeared in small quantities on Quirk's medium I, potato-dextrose agar, and Thaxter's agar.

All three color types showed up most strikingly on Quirk's medium II inoculated after leaching. The first to appear (in 18 hours) was an S chalk-white growth which gradually changed to RS and spread over the surface, the only type visible during the first 5 days. The 7th day this white layer was dotted with numerous small, raised S yellow and S pink colonies.

During the 34 serial passages covering a 2-years' association with the virus *in vivo*, some factor or factors had encouraged the development of the less infectious R-S opaque-white and the nonpathogenic S pink variants at the expense of the virulent S yellow type commonly recognized as *Xanthomonas phaseoli*. This "typical" virulent form was assuming a subordinate rôle and having difficulty in asserting itself.

"Plaques,"⁶ appeared in all beef-infusion agars inoculated before and after leaching, on potato-dextrose agar before leaching, and on Quirk's medium I after leaching.

Second lineal transfers from the aged plate of P(M + P)³⁴. Transfers were made to potato-dextrose agar, Thaxter's potato-dextrose agar, and pH 7.0 beef-infusion agar from each of the flasks of Quirk's media II, III, and IV, when the latter were 13 days old.

The P(M + P)³⁴ strain of *Xanthomonas phaseoli* still dissociated in these second lineal transfers from the aged plates.

The mildly pathogenic R-S white variant was the first to appear in all cultures of the nonleached series. The white form likewise developed first in the leached series with but two exceptions (on potato-dextrose and on Thaxter's agars, in transfers from Quirk's medium III) where only the "typical" S yellow form and a trace of S pink appeared. The R-S white variant was the only type to appear on potato-dextrose agar from Quirk's medium III in the nonleached series or on pH 7.0 agar from any source.

The virulent S yellow form, nevertheless, was beginning, in these second lineal transfers, to recover from the subordination so marked in the initial cultures made from the aged plate (Fig. 1. Series B and C). It was regaining its normal dominance to a greater extent in the leached than in the nonleached series.

⁶ Small clear areas resembling the plaques (bare spots) described by d'Herelle (7, p. 12, 20, and 95) as due to the lytic action of bacteriophage.

The S pink form was present in the leached set only. It appeared in cultures on potato-dextrose agar and on Thaxter's agar from all sources.

All three types appeared together in the potato-dextrose and the Thaxter's agars from Quirk's media II and IV in the leached series.

"Normal" strain. Transfers from the aged plate before and after leaching (Fig. 1, Series B and C, respectively). The "normal" *Xanthomonas phaseoli* was much less readily dissociated than was $P(M+P)^{34}$. The preliminary $\pm R$ white growth of the R-S white variant, so prominent in $P(M+P)^{34}$, appeared only in transfers made after leaching with the hot 0.5 per cent dextrose solutions though as the culture grew older some S white appeared on medium IV in the nonleached set. The R-S white form was conspicuous only in the flask cultures of Quirk's media II, III, and IV on which the dissociation phenomena are far better demonstrated than in the media most commonly used for bacterial plant pathogens. On Quirk's media II, III, and IV in the leached series there was an S white growth in 18 hours which was, however, quickly overrun by the "typical" virulent S yellow type so that in 5 days but little of the white growth was visible in any of the flasks. Ultimately it was covered completely. It was noted that the R-S white variant in this leached set of "normal" *X. phaseoli*, though overrun so quickly by the S yellow type, was apparently less subjugated by the latter on medium II than on media III or IV, as was shown by second lineal transfers.

In the tube cultures transferred either before or after leaching from the aged plates of Quirk's medium I, viz., to medium I, potato-dextrose agar, Thaxter's potato-dextrose agar, pH 6.0, pH 7.0, and pH 8.0 beef-infusion agars, there was a striking contrast between the S yellow growth of this "normal" *Xanthomonas phaseoli*⁷ and the $\pm R$ white growth of $P(M+P)^{34}$ (Fig. 2). Another notable difference between the two strains on these commonly used media was the much greater inhibiting effect of leaching on the "normal" *X. phaseoli*.

The S pink form appeared in "normal" *Xanthomonas phaseoli* only after filtering, a further contrast to $P(M+P)^{34}$. This was also true of second lineal transfers.

"Plaques" appeared less frequently than in $P(M+P)^{34}$ and then only after leaching, viz., on pH 6.0 and pH 7.0 beef-infusion agars.

Second lineal transfers from the aged plate of "normal" Xanthomonas phaseoli. Transfers were made from each of the flasks of Quirk's media II, III, and IV (when 13 days old) to potato-dextrose agar, Thaxter's potato-dextrose agar, and pH 7.0 beef-infusion agar.

Unlike $P(M+P)^{34}$, the "normal" *Xanthomonas phaseoli*, with a few outstanding exceptions, dissociated but little in these second lineal transfers from the aged plate. The R-S white variant failed to appear in the majority of transfers (both nonleached and leached sets) and occurred only as a trace in a few others. The few outstanding exceptions to this minor

⁷ There was some slight out-cropping of white on most solids at one time or another in the tube set of "normal" *X. phaseoli* made after leaching.

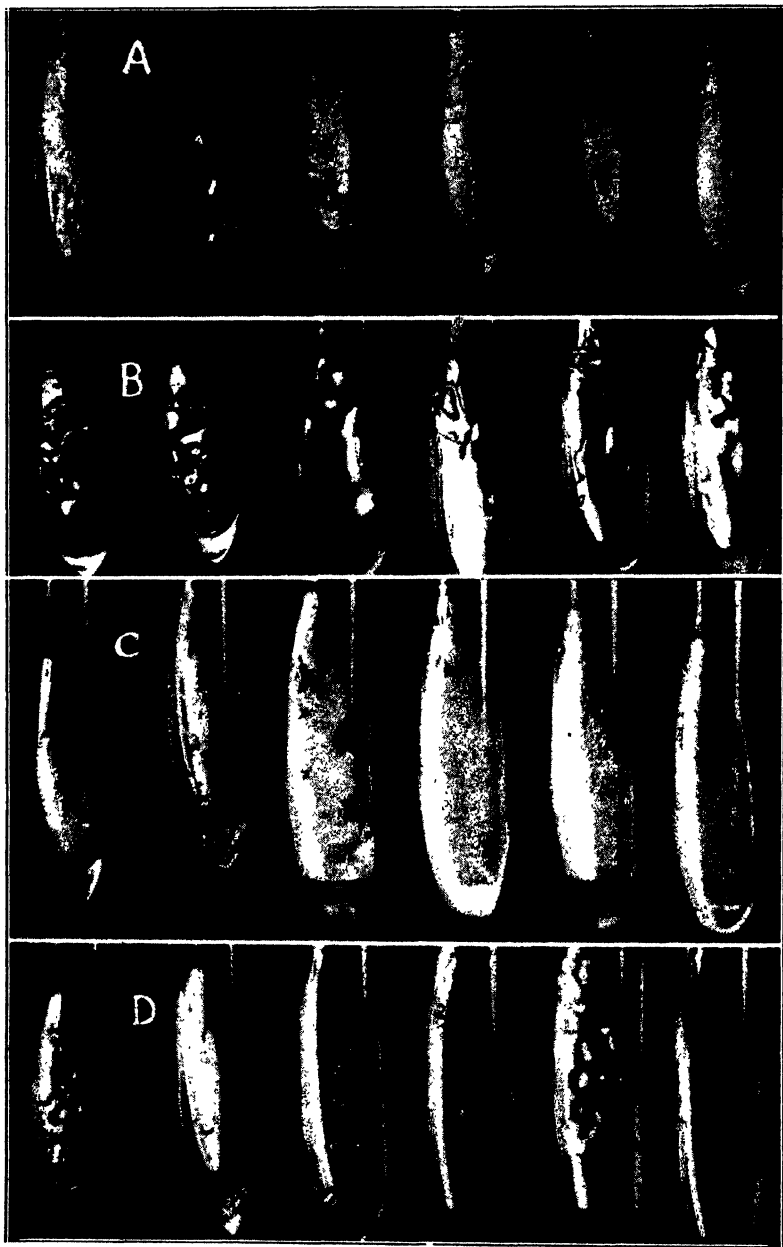


FIG. 2. Eleven-day-old transfers from aged plates of Quirk's medium I before and after leaching, showing difference between $P(M+P)^{24}$ (*X. phaseoli* associated continuously *in vivo* for 2 years with bean virus 1) and "normal" *X. phaseoli*.

Media, l. to right: Quirk's I agar, potato-dextrose agar, Thaxter's potato-dextrose agar, pH 6.0, pH 7.0, pH 8.0 beef-infusion agar. A. $P(M+P)^{24}$ cultured before leaching showing good \pm R white growth; trace of S yellow type-form in some. B. "Normal" strain cultured before leaching; good "typical" S yellow growth characteristic of the virulent type-form of *X. phaseoli*. C. $P(M+P)^{24}$ cultured after leaching; inhibition on potato-dextrose agar; good \pm R white growth on all others, trace of S yellow and S pink forms. D. "Normal" strain cultured after leaching; marked inhibition of S yellow growth; trace of whitish growth in some.

rôle of the mildly pathogenic R-S white form were on pH 7.0 beef-infusion agar from medium II in the nonleached set and the transfers to all three media from medium II in the leached set. In all four of these cultures, the R-S white form was dominant and, furthermore, it was the only one to appear in two of them. The dominance of the R-S white form in all three transfers from medium II leached was all the more noteworthy because at the time these transfers were made, the preliminary \pm R white growth in the parent flask culture had been completely overrun by the virulent S yellow type-form.

The virulent S yellow form was dominant in all other second lineal transfers, occurring in seemingly pure culture in the majority and growing as hitherto considered characteristic⁸ of *Xanthomonas phaseoli*. There was no trace of the S pink variant.

II. Behavior of Check Transfers from Young Cultures

At the same time that transfers of the two isolates, P(M + P)³⁴ and "normal" *Xanthomonas phaseoli*, were made from aged plates of medium I to flasks of Quirk's media II, III, and IV and to the tube setup of Quirk's medium I and the media most commonly used for bacterial plant pathogens, a parallel set of media was inoculated from young (3-day-old) steamed-potato cultures (Fig. 1, Series A). Both the undiluted bacterial growth and a suspension in pH 7.0 beef-infusion broth were used as inoculum. There was no leaching.

Both P(M + P)³⁴ and "normal" *Xanthomonas phaseoli* from young cultures produced, in 18 hours, the good S yellow growth characteristic of the virulent form of this organism. There was no trace of the preliminary chalk-white growth varying from R to S which was dominant in all P(M + P)³⁴ transfers from aged plates of Quirk's medium I and which, in the case of "normal" *X. phaseoli*, was visible for a short time only in flask transfers made to Quirk's media II, III, and IV from the aged plates after leaching.

As the transfers from young parent cultures grew older, some traces of a white form appeared in some of them as whitish streaks or patches, and on medium IV an infiltrating white form appeared. This was true of both "normal" *Xanthomonas phaseoli* and P(M + P)³⁴. No S pink form appeared in either strain.

In short, when young cultures were used as inoculum for a comparison of the two strains, the inherent differences between them were not evident on either the media most commonly used for bacterial plant pathogens or on Quirk's media I to IV.

III. Filtrates of Dissociated Cultures

Very much less difference between the strains P(M + P)³⁴ and "normal" *Xanthomonas phaseoli* with regard to dissociation was apparent in cultures

⁸ Copious syrupy S yellow growth on media rich in carbohydrates.

from the filtrates (Fig. 1, Series D) than was found between them when cultured after aging and leaching, but prior to filtering. In each strain a white or colorless form varying from R to S and, for the most part, but mildly infectious came through the filter. In some cases there was also an S yellow form.

In the second or third lineal transfers from the filter tubes (both strains), the S pink variant not infrequently put in an appearance. The pink form occurred in the form of superimposed colonies or as streaks or sectors in the white or greenish yellow S growth. The S pink variant was much more conspicuous in the P(M + P)³⁴ strain than in the "normal" strain.

Of special interest are the facts that:

1. The filtrate of neither strain produced growth in more than one of the commonly used culture media in the tube setup made directly from the filter tubes as soon as possible after filtering (Fig. 1, Series D). There was growth in pH 7.0 beef-infusion broth with P(M + P)³⁴ and in pH 6.0 broth with "normal" *Xanthomonas phaseoli*. On the other hand each strain produced growth of some kind on Quirk's media II, III, and IV in the flask setup of the same date.

2. The barely visible, finely roughened, film-like growth occurring on Quirk's medium II with P(M + P)³⁴ and on Quirk's media II and III with the "normal" strain was wholly unlike anything previously encountered with *Xanthomonas phaseoli*. Smears from the films on medium II (both strains) showed the presence of rods and cocci. Filaments also were present in the "normal" strain smears, some very long and branched, some segmented and breaking up. Such pleomorphism is characteristic of R forms in general and has been reported for many animal pathogens. Transfers from the P(M + P)³⁴ film produced clouding in pH 7.0 beef-infusion broth and an S greenish yellow growth with white margin on pH 7.0 beef-infusion agar. A pH 7.0 agar subculture of the last mentioned had striking S pink sectors in the S greenish yellow nailhead. No visible growth appeared in transfers from the P(M + P)³⁴ film to potato-dextrose agar or Thaxter's potato-dextrose agar.

Transfers from the "normal" strain film on Quirk's medium II produced an R colorless film on pH 8.0 beef-infusion agar but no visible growth on any other of the media most commonly used for phytopathogenic bacteria.

The film produced by the "normal" strain on Quirk's medium III was similar in appearance to those above mentioned but was interspersed with clear areas and consisted, at the time of examination, of spherical bodies (which may have been filaments broken up). It resembled ice thawing and breaking up on a lake. Transfers from this to a set of the most commonly used culture media produced an RS chalk-white growth on pH 6.0 beef-infusion agar, an R colorless film on pH 8.0 agar, and a scant growth of S colorless colonies on Quirk's medium I. There was no visible growth on other media.

3. The filtrate of the "normal" strain did not revert to the "typical"

S yellow form *in vitro* through aging in pH 6.0, pH 7.0, or pH 8.0 beef-infusion broths. The term "reversion" is used in this paper in the sense of a return to the type-form of *Xanthomonas phaseoli*. This might result (a) if individual cells in the "atypical" growth gave rise to progeny containing both "atypical" and "typical" forms of *Xanthomonas phaseoli* and the latter subsequently gained predominance or (b) because of the development of the "typical" form already present but masked in the "atypical" growth or from a combination of (a) and (b).

The transfers to the pH 6.0, pH 7.0, and pH 8.0 beef-infusion broths for aging were made on April 25 from the March 11 culture on medium IV when it was 6½ weeks old (see Fig. 6). At intervals up to the 76th day, transfers to a tube setup were made from these broth cultures with the following results:

Not even a trace of a yellow form came up in any transfer from the filamentous form of the R-S white variant in the pH 6.0 broth. Greenish yellow growth appeared in early transfers from pH 7.0 and 8.0 broths but by the 76th day the R-S white variant was dominant in subcultures of each. There was a trace of the S pink form in some transfers from each of the three broths. In other words there was at no time, in any of the subcultures of these broth transfers taken from the aged March 11 flask culture on medium IV, any growth resembling that of the "typical" virulent S yellow form of *Xanthomonas phaseoli*.

On the other hand, in 10 days there was reversion *in vivo* of the R-S white variant from the "normal" strain filtrate to "typical" S yellow *Xanthomonas phaseoli*. The inoculum was from a pH 7.0 beef-infusion agar transfer from the same March 11 flask culture on medium IV, but made when the latter was but 12 days old (Fig. 6, Inoc. 3).

4. After 10 days' incubation of the filtrate in the 0.5 per cent dextrose solution in the filter tubes,⁹ transfers to Thaxter's agar from both strains gave the characteristic S yellow growth of "typical" *Xanthomonas phaseoli*. In the P(M + P)³⁴ strain there were white patches of the R-S white variant as well.

BEAN PLANT INOCULATIONS

I. Dissociated Cultures Used as Inoculum Prior to Filtering

A. Flask cultures on Quirk's medium IV as inoculum.

Two-week-old cultures on Quirk's medium IV made from the aged plates of Quirk's medium I before and after leaching were used as inoculum on Stringless Green Refugee bean seedlings.

Results with the "normal" strain (Fig. 4, March 24 inoculations 1 and II). The "normal" *Xanthomonas phaseoli*, both before and after leaching, was extremely virulent, markedly more so than was P(M + P)³⁴. It worked more rapidly and caused a much more severe type of bacterial infection. The difference was particularly marked in the secondary infection on the

⁹ After the initial transfers, the tubes of filtrate had been kept under observation. In 10 days the "normal" filtrate was still clear, that of P(M + P)³⁴ clouded.

stems where, with the "normal" strain, there was much external discoloration in 28 out of 29 ($96\frac{1}{2}$ per cent) of the plants inoculated March 24; and 22 of them had bacterial ooze. The pathogenicity of the "normal" strain was little affected by the leaching, probably because the less virulent R-S white variant which came up in the young cultures was so quickly overrun by the S yellow type-form of *N. phaseoli*.

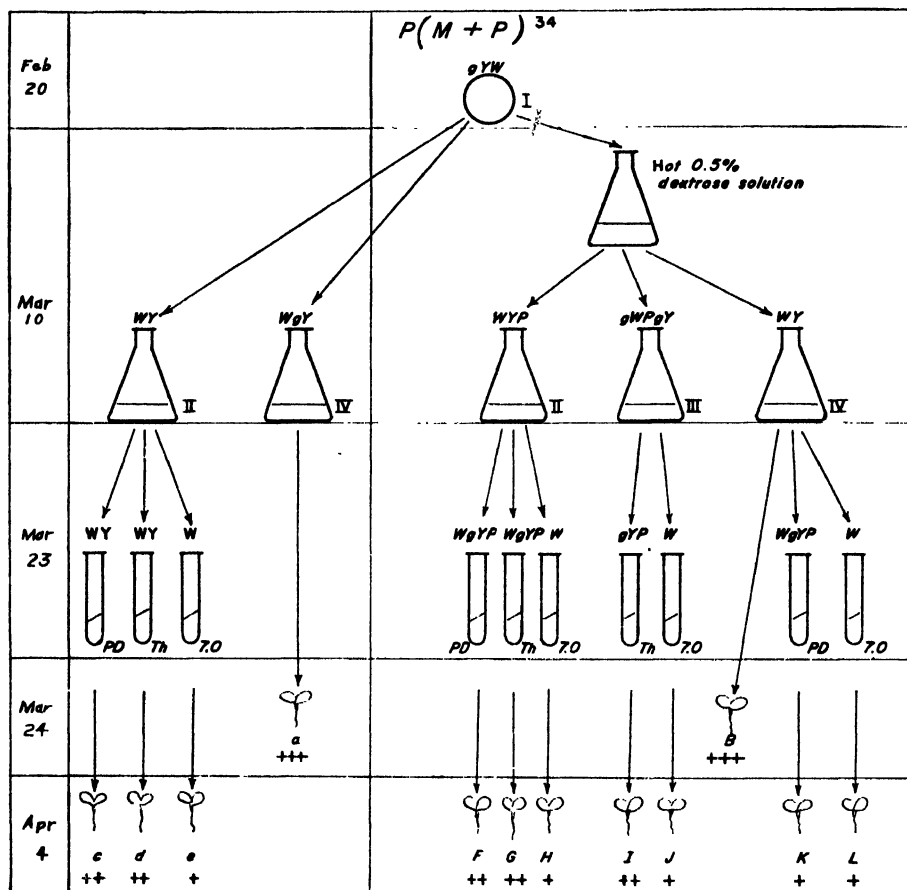


FIG. 3. Inoculations of bean plants with dissociated $P(M + P)^{34}$ before and after leaching, showing ancestry and color of cultures used as inoculum. Plus signs are qualitative rather than quantitative symbols of bacterial infection.

Abbreviations: Media: I-IV, Quirk's potato media; PD, potato-dextrose agar; Th, Thaxter's potato-dextrose agar; 7.0, pH 7.0 beef infusion agar. Color of growth: P, pink; W, white; Y, yellow; w, trace white; y, trace yellow; gW, greenish-white; gY, greenish-yellow.

This greater virulence of the "normal" strain was to be expected since the dissociation of the two strains had shown that the "normal" contained a much greater concentration of the highly virulent S yellow form than did $P(M + P)^{34}$. No seed infection was observed in the checks.

Results with $P(M + P)^{34}$ (Fig. 3, March 24 inoculations a and B). Cultures of $P(M + P)^{34}$ on Quirk's medium IV produced, like those of the nor-

mal strain, 100 per cent bacterial infection, both before and after leaching, but though this was good to excellent on the inoculated primary leaves, the secondary infection on the stems was very mild. There was also evident in this strain, a marked lessening of the invasion of stem tissues in the plants inoculated with cultures in the leached series. This was not surprising inasmuch as a study of the dissociated cultures had shown that the leaching

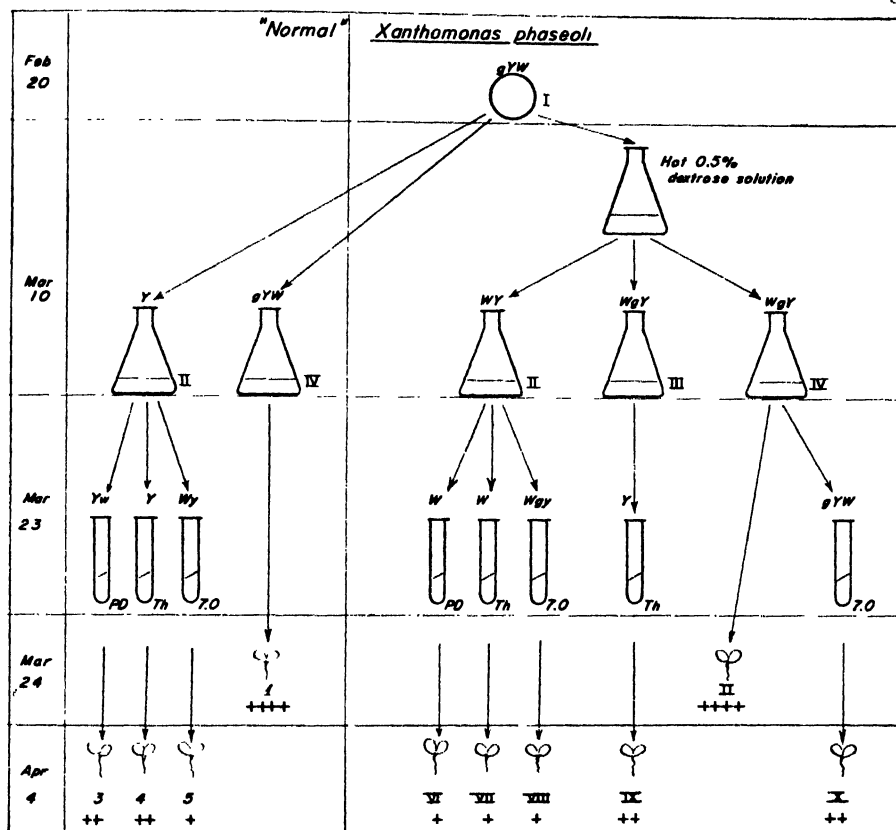


FIG. 4. Inoculations of bean plants with dissociated "normal" *Xanthomonas phaseoli*. All data parallel those in figure 3.

had further favored the weakly-pathogenic or avirulent variants, R-S white and S pink, seemingly already encouraged by the 2 years' association with the virus *in vivo*. The majority of the plants inoculated with P(M + P)²⁴ before leaching showed slight but distinct stem discoloration and some internal browning. On the contrary, of those inoculated with leached P(M + P)²⁴, 14 out of 22 (64 per cent) showed no external or internal sign of stem infection though microscopic examination revealed the presence of bacteria in the stem tissues of 21 of the 22. Bacterial ooze was present in but 4 of the 33 plants inoculated March 24, 2 out of 11 in the nonleached series, and 2 out of 22 in the leached series. No seed infection was observed in the checks.

B. *Subcultures from initial flask cultures as inoculum (Second lineal transfers from aged plates of strain P(M + P)³⁴ and "normal" strain).*

No direct inoculations were made from flask cultures on Quirk's media II and III from the aged plates of the two strains before and after leaching, but 15 selections from 12-day-old transfers from said flask cultures (when two weeks old) to potato-dextrose, Thaxter's potato-dextrose, and pH 7.0 beef-infusion agar (Figs. 3 and 4, March 23 cultures) were tested for pathogenicity on 2-week-old Stringless Green Refugee bean seedlings.

On the same date, 12-day-old transfers from flask cultures on medium IV from the aged plates (both strains; leached series) were tested on bean plants for comparison with the March 24 inoculations with the parent cultures on medium IV *per se* recorded above (Section A).

Results of inoculation (both strains, Figs. 3 and 4, April 4 inoculations).

The difference in results obtained with the two strains was on the whole much less marked in these inoculations with the second lineal transfers than in those described in Section A made with the initial flask cultures on medium IV transferred directly from the aged plates (Figs. 3 and 4, March 24 inoculations). All the second lineal transfers from the aged plates of each strain produced bacterial infection, although in some sets there was no external sign of primary infection on the rubbed leaves or of secondary infection on the stems (both strains); the plants in such cases were believed to be healthy until microscopic examination revealed the presence of bacteria in the stems. Such masked infection occurred in 4 sets of inoculations with the "normal" strain (Fig. 4, April 4 inoculations 5, VI-VIII) and in 2 of the P(M + P)³⁴ sets (Fig. 3, April 4 inoculations e and J). It ranged from 16½ to 60 per cent of the plants inoculated with "normal" and from 35 to 66½ per cent with P(M + P)³⁴. (The plus signs in figures 3 and 4 are qualitative rather than quantitative symbols.)

Though the 18 transfers were pathogenic in varying degrees (both strains) none was sufficiently virulent to cause severe leaf infection, premature killing of primary leaves, or girdling, splitting, or breaking of the stems. Where the R-S white variant was dominant in either strain (more often than not on pH 7.0 beef-infusion agar), there was mild or poor, usually masked, infection. Sometimes the signs of disease were confined to barely perceptible discoloration of a single vessel in the stem. There was always more or less good infection with either strain wherever there was a fair amount of the S yellow form in the culture used as inoculum.

There was no obvious effect of the S pink form on the virulence of the S yellow form.

The subcultures from Quirk's medium IV leached (both strains) were much less virulent (Figs. 3 and 4, April 4 inoculations K, L, and X) than had been those from the parent flasks *per se* (Figs. 3 and 4, March 24 inoculations B, II). This decrease in virulence in the second lineal transfers was more pronounced with P(M + P)³⁴. The subcultures from medium IV leached (both strains) had been made the day preceding the use of the

2-week-old parent flasks as inoculum on March 24, and were themselves used for inoculation at approximately the same age.

No evidence of seed-borne infection (bacterial or virus) was observed in the large number of noninoculated checks nor did microscopic examination of these healthy appearing plants reveal the presence of bacteria.

II. *Filtrates of Dissociated Cultures Used as Inoculum*

With the filtrate of each strain grown on various media, bacterial infection, mild for the most part, was produced on Stringless Green Refugee bean seedlings (Figs. 5 and 6). No culture was wholly avirulent.

With a single exception, no visible leaf infection was produced with the filtrate of either strain of the organism when the inoculum consisted of (a) any of the initial transfers of March 11, (b) subcultures of the same, or (c) the contents of the filter tube *per se* after 10 days' incubation.

In the exceptional case cited (Fig. 6, April 4 inoculation 3 with pH 7.0 beef-infusion agar subculture of the "normal" strain on medium IV) the R-S white variant produced inconspicuous water-soaking on the rubbed primary leaves and mild stem infection in 40 per cent of the plants. Isolation plates from the watersoaked spots on one of the rubbed leaves 10 days after inoculation, showed that the R-S white variant had, for the most part reverted in the host to "typical" S yellow *Xanthomonas phaseoli*. There were innumerable S yellow colonies and but 4 colonies of the R-S white form. At the time it was used for inoculum, the R-S white variant had been making a + S growth and the 4 colonies mentioned were S white.

Stem infection was produced by all the filtrate cultures although in some cases (both strains) there were little or no external signs of this. However, microscopical examination revealed bacteria in 9 to 100 per cent of these seemingly healthy plants. (Plus signs in figures 5 and 6 are qualitative rather than quantitative symbols.)

The only severe infection on both leaves and stems (Figs. 5 and 6, May 16, inoculations 8 and 9) was produced by potato cultures (both strains) descended via Thaxter's potato-dextrose agar, from the bacterial suspension in the filter tubes¹⁰ incubated 10 days, during which time the "typical" virulent S yellow form of *Xanthomonas phaseoli* had developed. There was 100 per cent excellent infection on the rubbed primary leaves, with girdling, splitting, and breaking over of the stems accompanied by extrusion of bacterial slime. It is interesting to note by way of contrast that inoculations made directly from the bacterial suspension in the 10-day-old filter tube (both strains) had produced no visible infection on the leaves and only mild symptoms on the stems although 100 per cent of the plants inoculated with P(M, P)³⁴ (filter tube clouded) and 40 per cent of these inoculated with "normal" *X. phaseoli* (filter tube still clear) were infected (Figs. 5 and 6, March 20 inoculations a and 1).

Plates poured from one of the severely infected stems from the

¹⁰ The identical filter tubes had been used for the initial transfers.

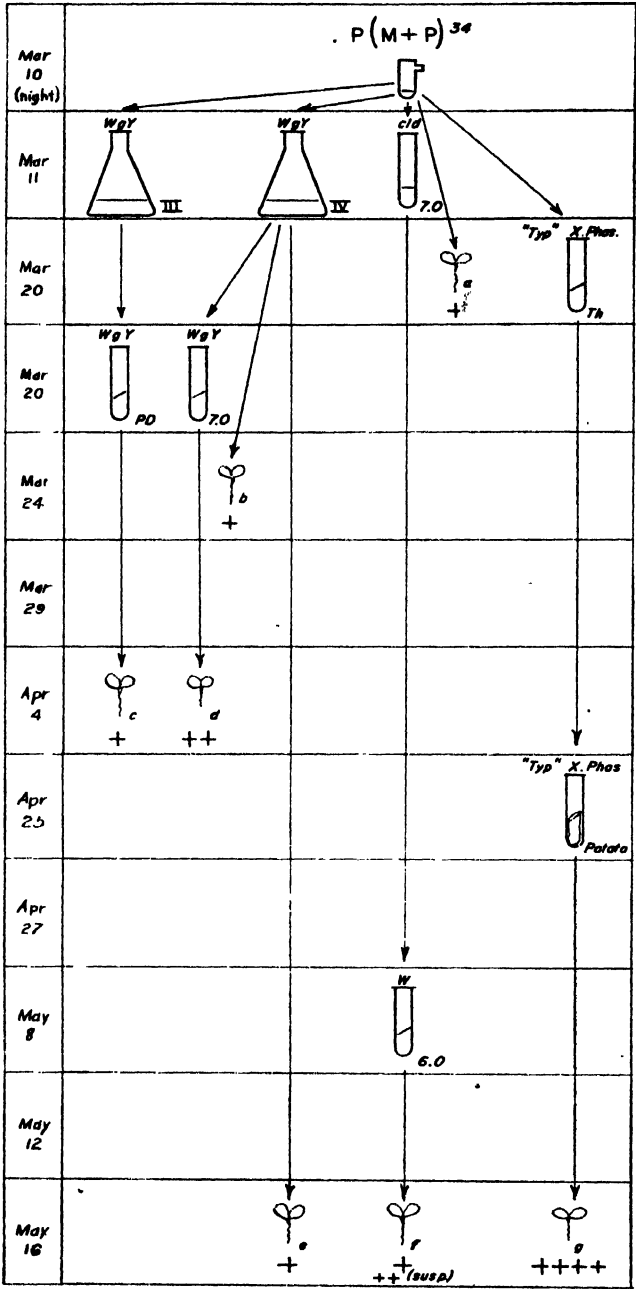


FIG. 5. Inoculations of bean plants with filtrates of leached plate culture of $P(M+P)^{34}$ aged on Quirk's medium I, showing ancestry and color of cultures used as inoculum. Plus signs are qualitative rather than quantitative symbols of bacterial infection.

Abbreviations: Media: III and IV, Quirk's potato media; PD, potato-dextrose agar; Th, Thaxter's potato-dextrose agar; 6.0, 7.0, 8.0, pH 6.0, pH 7.0, pH 8.0 beef-infusion agars or broths; Susp., sterile-distilled-water suspension of bacteria. Growth: Cld., clouded; fl., filaments; W, white; Y, yellow; pW, pinkish white; gY, greenish yellow; y, trace yellow.

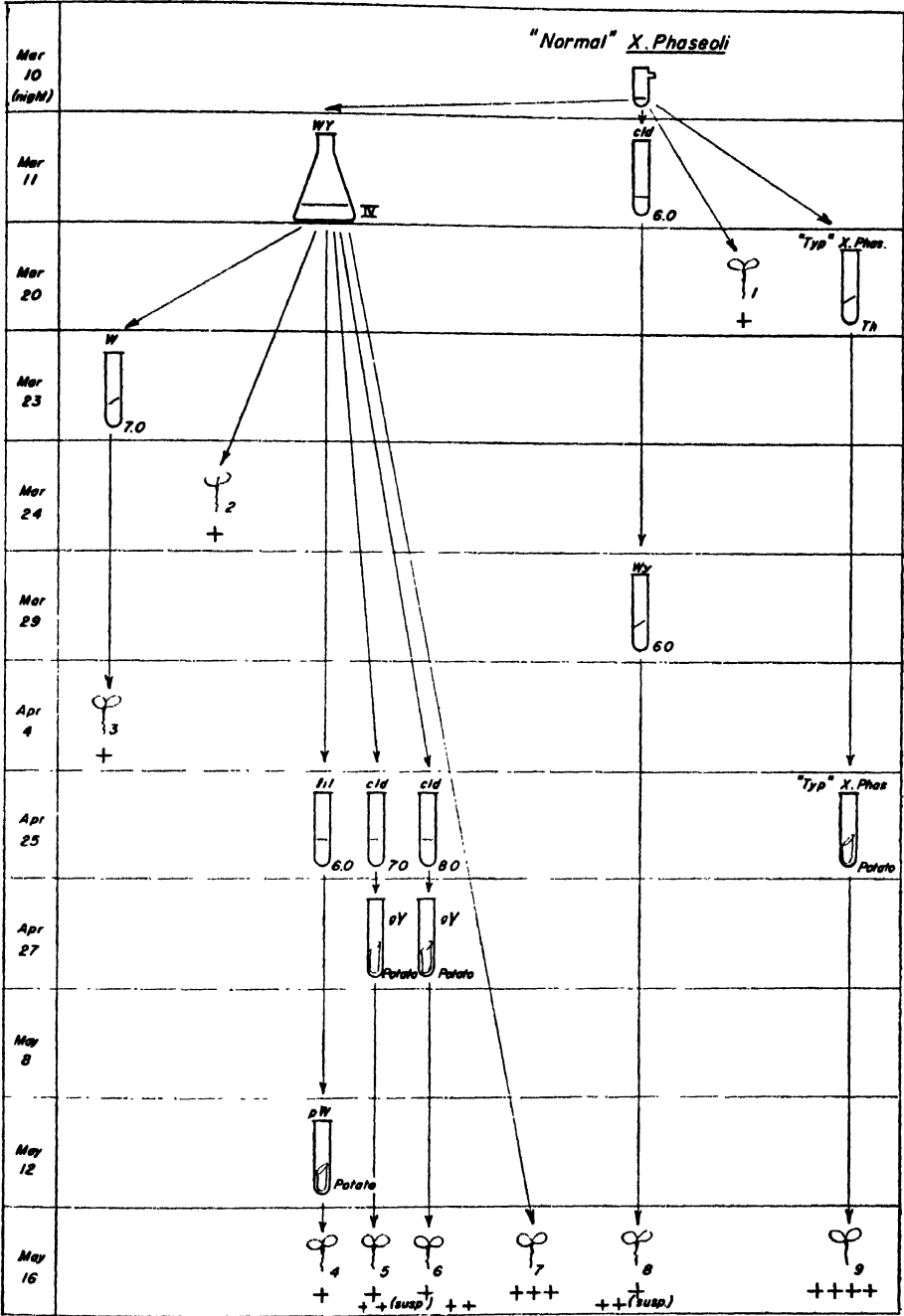


FIG. 6. Inoculations of bean plants with filtrates of leached plate culture of "normal" *Xanthomonas phaseoli* aged on Quirk's medium I. Reversion of R-S white form to "typical" S yellow *X. phaseoli* occurred in inoculation No. 3; April 4. All data paralleled those of figure 5.

P(M + P)³⁴ inoculation set g, contained both "typical" *Xanthomonas phaseoli* and the mildly pathogenic yellow variant of *X. phaseoli* described by the writer in an earlier paper (5, p. 665 and footnote 12). The variant was much in the majority.

Mosaic was produced in 100 per cent of the plants inoculated with non-diluted bacterial growth from the aged (2 months) initial flask culture on Quirk's medium IV of the filtrate of P(M + P)³⁴ (Fig. 5, May 16 inoculation e). There were no macroscopic signs of bacterial infection. Unfortunately only 6 plants were included in this set. The 11 plants inoculated at the same time with a suspension of bacteria from the same flask culture showed no symptoms of either mosaic or bacterial infection but microscopical examination revealed masked bacterial infection in 36½ per cent of them.

In the comparable inoculation of 15 plants with the "normal" strain filtrate (Fig. 6, May 16 inoculation 7) there was 100 per cent severe bacterial infection causing breaking over and killing of stems but no visible leaf lesions. Among the 11 plants inoculated with the bacterial suspension, 4 showed mosaic symptoms.

In the large number of noninoculated check plants no sign of seed-borne infection, bacterial or virus, was observed nor were masked bacteria revealed by microscopical examination of these healthy-appearing plants.

DISCUSSION

The P(M + P)³⁴ culture used for the dissociation studies originated from a single colony of *Xanthomonas phaseoli*. It was transferred from an isolation plate containing but 5 colonies and these well distributed. The "normal" strain likewise originated from a single colony in a thinly-sown plate. The colonies of the 2 strains were indistinguishable in appearance as were also the young cultures on steamed potato cylinders used as source inoculum for the dissociation experiments.

The conditions prevailing in the much stunted bean plants of the 34th serial passage of the 2 infective agents from which P(M + P)³⁴ was isolated were such as those described by Riker (12) as being particularly conducive to variation in bacterial plant pathogens, namely those "preventing rapid vegetative growth."

That variants existed in the serial passages had previously been shown by isolation plates as reported in an earlier paper (5). It was presumable, therefore, that the bacterial population in these dwarfed plants of the 34th serial passage of the associated infective agents was a complex of variants of *Xanthomonas phaseoli* and that, furthermore, these might be masked in colonies outwardly indistinguishable from those of "normal" *X. phaseoli*.

The object of these studies was to determine whether a colony transfer of P(M + P)³⁴ when subjected to a dissociation technique would differ from one of "normal" *Xanthomonas phaseoli* in a way which might explain the subsequent decrease in pathogenicity of the bacterial pathogen in the serial passages.

What variation the progeny of single cells may undergo is another problem and one which it is hoped will be undertaken by other investigators. No generalization regarding the variation of *Xanthomonas phaseoli* could have been made, however, from single-cell studies of P(M + P)³⁴, as it is well known that strains of bacteria vary greatly in their ability to dissociate and there is great need for the study of many strains before it will be possible to determine the norm for a given organism in regard to variation. Furthermore the progeny of different single cells may differ one from another. Hence the study of the progeny of many single cells from each of the many strains would be necessary to complete the picture.

It is interesting to note in this connection that a number of investigators have shown that single cells may produce unlike progeny. For example, Bengsten (1), working with organisms connected with botulism, demonstrated that the progeny of a toxic single-cell culture of type C (the Saunders' strain isolated from fly larvae) contained both toxic and nontoxic individuals. The progeny of nontoxic single cells, on the other hand, never gave rise to toxin-producing cultures. Quirk (10) found that by pH control she could produce pure plate-cultures of virulent S or avirulent R forms of *Bacillus phytophthorus* from single-cell isolates of either type. McNew (8), working with the progeny of a single cell of *Bacterium stewartii* E. F. Sm. isolated by the Riker *et al.* hanging-drop method, found that weakly virulent strains developed from highly virulent cells in nutrient-dextrose broth at 25–27° C. and gradually replaced the parent type. He states that cultures containing weakly virulent cells were not appreciably attenuated unless the proportion of highly virulent parental strains fell below 21 per cent.

Sectors indicating a sudden change from the parent culture, have been observed in single-cell cultures by various workers. For example, Nirula (9), working with an unidentified organism, found in several single-cell cultures wedge-shaped sectors from which he was able to separate strains markedly different from the parent cultures. These variations remained constant and reversion could not be induced by special methods of culture. More recently Elliott and Robert (3) found sectoring in nearly all of the colonies in potato-dextrose-agar plates poured from one of the progeny of a weakly virulent single-cell culture of *Bacterium stewartii*. In this case the variation appeared to be for color only.

The filtration of P(M : P)³⁴ and "normal" *Xanthomonas phaseoli* covered a longer period than is customary in filtration studies. This step (Fig. 1, Series D) was not reached till late afternoon and as filtration was carried on by gravity rather than by suction, in order to avoid the possibility of pulling the bacteria through the filter, insufficient filtrate had been obtained by nightfall to make the necessary number of transfers. Hence, the filtration setup was put into a refrigerator held at 10° to 12° C. and the transfers were made early the next morning. At this temperature *Xanthomonas phaseoli* could not have grown through the filter overnight.

White variant. The white variant of *Xanthomonas phaseoli*, varying from R to S, is the one that, in the writer's experience, has been the most commonly encountered both *in vitro* and *in vivo*. The same culture may be R or S or RS at different stages of its development. It has shown marked pleomorphism in certain phases of the variation process, as is commonly reported for R forms in general. It has been most markedly brought to the fore, particularly in P(M + P)¹¹, by Quirk's dissociation technique with her media I to IV, but has now and then been observed as a secondary growth in old steamed-potato cultures of a number of strains of *X. phaseoli* in addition to those discussed in this paper. Colonies of the R-S white variant have appeared from time to time in years past in pH 7.0 beef-infusion-agar plates, from plants naturally infected with *X. phaseoli*. These colonies occurred much less frequently and in smaller numbers in plates poured from such plants, however, than in those from plants inoculated in the writer's association studies of *X. phaseoli* and bean virus 1 previously described (5). In the latter studies, colonies spectacularly dissociating into white and yellow forms also were isolated at times from serial passages of bean juice containing the two infective agents (5, Fig. 2).

The ability of the R-S white variant to revert in part¹¹ to the "typical" S yellow *Xanthomonas phaseoli in vivo*, as noted in pathogenicity tests of the filtrates of the dissociated "normal" strain, was corroborated in later unpublished studies of another strain, "Wyoming 1940," both with cultures dissociated by Quirk's technique and with potato cultures from a series in which the R-S white variant had arisen spontaneously and superseded the S yellow one. The recurrence in the writer's (M + P) serial passages (5) of bacterial symptoms on the inoculated primary leaves after an absence for 10 such passages is believed to be due to such reversion of the R-S white variant.

Reversion also takes place *in vitro*, though less readily in the writer's experience. An occasional S opaque-white colony of the R-S white variant produced "typical" S yellow growth on steamed potato and excellent S white growth on pH 7.0 beef-infusion-agar in transfers made on the same date. In such cases the medium appears to exercise a selective action on the types occurring in the colony.

Though the R-S white variant is usually but mildly pathogenic, the writer on one occasion encountered in this form of the "Wyoming 1940" strain a phase in the early stage of its transition from the S yellow type-form when it was extremely virulent and did not revert to S yellow in the host. At the opposite end of the pathogenicity scale of this white variant of *Xanthomonas phaseoli*, Wyoming-1940 strain, was a phase which has been completely avirulent in the writer's studies. It forms \pm R colonies of the medusa-head type consisting of filaments (unpublished work). The writer (5) has isolated similar colonies from the serial passages of (M + P).

¹¹ There were always, in addition to the "typical" S yellow colonies, some of the R-S white variant present in the isolation plates from plants wherein reversion had occurred.

Unlike the starch-loving¹² S yellow type-form, the R-S white variant is very erratic in its reaction to steamed potato cylinders as a medium and frequently fails to grow at all thereon.

The studies described in this paper show that variants, both weakly pathogenic R-S white and nonpathogenic S pink, were very much more readily dissociated from the parent colony in the case of strain P(M + P)³⁴ than in that of the "normal" strain of *Xanthomonas phaseoli* used for comparison. In the dissociated cultures of P(M + P)³⁴ the mildly pathogenic R-S white variant was dominant. In the dissociated cultures of the "normal" strain on the other hand, the dominance of the "typical" virulent S yellow form was very marked. Was this dominance of the weakly pathogenic R-S white form in P(M + P)³⁴ due to the direct action of the virus upon the bacterium during their association *in vivo* or to its indirect influence through the stunting and physiological changes it caused in the host, conditions conducive to the production of variation in bacterial plant pathogens; to the mere presence of the virus and its competition for nutrient substances; in part to continuous plant passage *per se*¹³; or to a combination of factors?

Until we know more about the mechanism which gives rise to the results observed in bacterial or bacteria-and-virus associations, and understand more clearly the fundamental biological nature of the phenomenon of variation in the bacterial cell *per se*, we can do little more than speculate as to the answer. Too many unknown factors are involved.

Studies by Hedges and Fisher (6) relative to the effect of nutrient solutions containing varying amounts of nitrogen on the pathogenicity of the bacterium and the virus *in vivo*, either alone or associated in serial passages, showed that under the conditions of those experiments, the amount of N optimum for plant growth was optimum for both infective agents.

The demonstration of mildly pathogenic and avirulent variants of *Xanthomonas phaseoli* calls attention to the fact that the pathologist or plant breeder has solved but a part of his problem if he takes into consideration only the so-called "normal" type of this pathogen. Furthermore, the influence of certain culture media in bringing one or another form of the parasite to the fore emphasizes the importance of the medium as a factor which often affects stability of a given type of organism under cultural conditions.

SUMMARY AND CONCLUSIONS

These dissociation studies of *Xanthomonas phaseoli* were undertaken for the purpose of discovering whether changes had taken place in the bacterium during its 2 years' association with the virus *in vivo* (4, 5) which

¹² Evidenced by its luxuriant growth on steamed potato cylinders and its strong diastatic action.

¹³ Parallel tests of 3 strains of *X. phaseoli* in serial passages from bean plant to bean plant of juices containing the respective isolates, have produced no diminution in severity of symptoms of bacterial infection to date, after 16 serial passages covering 6½ months (unpublished work).

might account for its decrease in virulence and failure to maintain continuously its "typical" form in the host tissues. The writer hoped furthermore to determine whether there existed a filterable form of *Xanthomonas phaseoli* which was capable of producing mosaic.

Quirk's technique of dissociation was employed involving the use of her special media I to IV (11) and including the media commonly used for phytopathogenic bacteria. Check transfers to all media were made from young cultures.

Dissociated cultures manifested striking differences between a "normal" strain of *Xanthomonas phaseoli* and strain P(M + P)³⁴, a strain associated continuously for 2 years *in vivo* with the virus of the common bean mosaic. P(M + P)³⁴ had been isolated from the 34th serial passage of infected juice from bean plant to bean plant, *i.e.*, shortly before (a) the disappearance of all bacterial symptoms from the inoculated plants, which occurred in the 36th serial passage, and (b) the sudden onset of ultra-severe mosaic in the 38th serial passage (5).

The variations proved to be for color as well as for pathogenicity and R and S. In the dissociated cultures of P(M + P)³⁴, the mildly infectious R-S opaque-white and nonpathogenic S pink variants developed at the expense of the "typical" virulent S yellow form. This was best demonstrated in Quirk's media II and III. In the dissociated "normal" strain, on the other hand, with the exception of a trace on medium IV in the nonleached set the weakly pathogenic R-S white variant appeared only after leaching and was quickly overrun by the S yellow form. The S pink variant appeared in the "normal" strain only after filtering. The dissociation phenomenon and the differences between the two strains were far better demonstrated on Quirk's media II, III, and IV than on the media most commonly used for phytopathogenic bacteria.

The initial transfers of the filtrates of the leached, aged, medium-I plate cultures of each strain (Fig. 1, Series D) produced growth on Quirk's media II, III, and IV. Transfers made at the same time to 9 media commonly used for bacterial plant pathogens resulted in growth in two only, both beef-infusion broths, *viz.*, pH 6.0 inoculated with "normal" *Xanthomonas phaseoli* and pH 7.0 with P(M + P)³⁴. In all subcultures from the two strains in these respective broths the R-S white variant was dominant.

A barely visible, colorless, finely roughened, film-like growth was produced on medium II with the filtrate of each strain, and on medium III with that of the "normal" strain. Transfers from such films grew on a few media. Each strain produced on medium IV an initial S growth of the R-S white variant followed by the S yellow form in the case of the "normal" strain or by the S greenish yellow form with P(M + P)³⁴. P(M + P)³⁴ produced a similar growth on medium III. An S pink variant appeared in second or third lineal transfers from the bacterial suspension in the filter tube in each strain. It occurred in the form of superimposed colonies, streaks, or sectors, and it was much more conspicuous in the P(M + P)³⁴ strain.

The "typical" virulent S yellow form was recovered from the filtrate of each strain when transfers to Thaxter's potato-dextrose agar were made from the filter tube after 10 days' incubation. In the case of P(M+P)³⁴ this was accompanied by patches of S growth of the R-S white variant.

Inoculations of Stringless Green Refugee bean seedlings with the dissociated cultures of each strain showed that none of those tested, including filtrates, was wholly avirulent. The severity of infection was directly proportional to the amount of the S yellow form in the inoculum. When the R-S white variant was dominant infection was poor or only fair, rarely visible on the inoculated leaves, and more often than not revealed only by microscopic examination of the stem. The R-S white variant from the "normal" strain filtrate reverted to "typical" S yellow *Xanthomonas phaseoli* in the host within 10 days after its use as inoculum.

Some mosaic appeared in inoculations with aged, medium-IV flask cultures of the filtrates of each strain. However, although the large number of inoculated checks showed no sign of seed infection, more work would need to be done and with a greater number of plants to prove conclusively that a filtrate of *Xanthomonas phaseoli* culture was capable of producing mosaic.

The writer concludes that the dissociation studies explain the decrease in virulence of *Xanthomonas phaseoli* during its 2 years' *in vivo* association with the common bean-mosaic virus by demonstrating the increased development of the weakly pathogenic R-S white and nonpathogenic S pink variants, which resulted in the partial subordination of the virulent S yellow type-form of the bacterium.

The writer believes that the properties of virulence, mild virulence, and avirulence are inherent in all so-called "typical" cultures of *Xanthomonas phaseoli* and that the dominance of one or another type may be determined by a number of factors both *in vitro* and *in vivo*. In this connection it seems quite probable that an associated infective agent, if present, may play, either directly or indirectly, an important rôle.

In studies such as those reported in this paper there are, in the opinion of the writer, a number of problems that require for their solution the collaboration of pathologist, bacteriologist, virologist, biochemist, geneticist, and physicist.

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ASSOCIATION OF *XANTHOMONAS PHASEOLI* AND THE
COMMON BEAN-MOSAIC VIRUS, MARMOR *PHASEOLI*.
III. THE EFFECT OF VARYING AMOUNTS OF
NITROGEN ON PATHOGENICITY

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INTRODUCTION

Earlier studies by the senior author (2, 3) demonstrated that the virus symptoms predominated in serial passages from bean plant to bean plant of bean juice containing *Xanthomonas phaseoli* (E. F. Sm.) Dowson and the virus of the common bean mosaic, *Marmor phaseoli* Holmes. The mosaic symptoms suddenly assumed an ultra-severe form in the 38th serial passage, after two years' uninterrupted association of the virus with the bacterium in the host. Bacterial symptoms on the other hand varied from none or very mild signs of infection to good typical *X. phaseoli* lesions. No severe secondary bacterial infection occurred at any time.

Very virulent isolates of *Xanthomonas phaseoli* were obtained from time to time by means of poured plates from the serial passages as well as colonies spectacularly dissociating into the "typical" S yellow form and the mildly pathogenic white variant. (3, figure 2.) At times, notably in plates from plants with an ultra-severe form of the mosaic, no "typical" *X. phaseoli* appeared. There were, however, aberrant forms which the senior author's later dissociation studies (4) of a virulent isolate from the serial passages led her to conclude were variants of *X. phaseoli*.

That virus infections cause considerable disturbance of the physiological processes is well known (17). The common bean mosaic virus is no exception to this general rule. It is not strange, in view of this fact, that *Xanthomonas phaseoli* did not find good growing conditions in such host plants as those described in the serial passages (3).

Various investigators have reported on the effect of nitrogen on both virus and bacterial infections, *e.g.*, Spencer (9, 10, 11, 12, 13), working with tobacco-mosaic virus, found a direct correlation between virus activity and the amount of nitrogen furnished the plants and none between the growth of the host as influenced by the nitrogen supply and the increase in virus content. In other words, an excess of nitrogen beyond that producing maximum growth of the host caused maximum susceptibility.

Woods and Du Buy (16) concluded that sufficiently prolonged nitrogen starvation could prevent or retard multiplication of the tobacco-mosaic virus by reducing the activity of cyanide-sensitive respiration on which said multi-

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plication depends. The work was done with tobacco seedlings grown in sand cultures.

Considerable work has been done by McNew (6, 7) and McNew and Spencer (8, 14) on the effect of nitrogen on *Bacterium stewartii* E. F. Sm. It was found that the maximum severity of infection with this pathogen occurred with excess nitrogen and that there was a tendency for virulent strains to be replaced by attenuated ones in seedlings rendered insusceptible by nitrogen deficiency. The ability of a strain to use inorganic nitrogen was essential to its virulence, weakly virulent strains being obligate users of organic nitrogen (7), and there was a stronger tendency for the development of virulent strains in nitrogen-fed plants than in those deficient in nitrogen.

Clayton (1) found that high nitrogen fertilization combined with low topping of tobacco plants, a cultural practice in some regions, favored maximum development of wildfire by increasing susceptibility to water-soaking which he found to be a necessary condition for the occurrence of the destructive epidemic type of wildfire. Experimental field plants with low nitrogen fertilization were difficult to water-soak and were resistant to *Pseudomonas tabaci* (Wolf and Foster) Stapp in both sandy and more fertile soils.

A comprehensive review of the work of numerous investigators on the effect of nutrition on the susceptibility of plants to disease has been published by Wingard (15).

The senior author's earlier work (3) had been carried on with pot experiments using sterilized composted soil. The present investigations were undertaken with the purpose of studying the effect in sand cultures of varying amounts of nitrogen on the disease complex and on the activity of the two infective agents *per se*. A preliminary report has been published (5).

MATERIALS AND METHODS

Bean seedlings were grown in sterilized, washed quartz sand and thinned to 3 plants per 4-inch pot in experiment I, to 4 plants in experiment IV. Later 6-inch pots with 4 plants each were used. The pots were supplied with saucers.

The inocula consisted of (a) the crushed tops of infected plants from the serial passages of bean juice containing the bacterium and the virus, (b) pure single colony cultures of *Xanthomonas phaseoli* growing on steamed potato cylinders, and (c) crushed infected trifoliate leaves or plant tops from serial passages of bean juice containing the mosaic virus.

The inoculum was rubbed on the under surface of primary leaves when they were $\frac{1}{2}$ to $\frac{3}{4}$ full grown, at which time the seedlings were usually about 10 days old and no trifoliate leaves had unfolded. No carborundum was used.

Feeding of the sand cultures began one or two days after inoculation and consisted of the addition of 100 ml. nutrient solution to each pot 6 times weekly. The plants were watered with tap water as needed.

The nutrient solutions containing low, medium, and high nitrogen were

made up according to the formulae published by Spencer (11, p. 770) in his paper on the effect of host nutrition on concentrations of tobacco-mosaic virus. The solutions contained 9.8 p. p. m., 200 p. p. m., and 2000 p. p. m. nitrogen, respectively.

For comparison, inoculations were made on plants growing in sterilized composted soil.

Noninoculated plants as checks on seed infection were grown both in sterilized sand watered with the nutrient solutions and in sterilized composted soil. They were kept isolated from the inoculated plants.

The line of Stringless Green Refugee bean used in these investigations is the same as that employed in all previous association studies by the senior author (3, 4). The seed was collected by W. J. Zaumeyer from healthy plants in his breeding plots in Greeley, Colorado, or from the writer's healthy non-inoculated checks from the same seed source. The same is true of U. S. No. 5 Refugee.

Abbreviations

M = bean mosaic virus.

P = *Xanthomonas phaseoli*.

P. var. = a yellow variant of *X. phaseoli*, differing from the "normal" strain in colony characters (3, p. 665, footnote 2), growth on steamed potato, and pathogenicity when first isolated. It usually reverts to the "normal" form in later passages through the plant. The strain of P. var. used in these fertilizer experiments was isolated 4 years previously from the petiole of a trifoliate leaf of Stringless Green Refugee inoculated by rubbing the primary leaves with *X. phaseoli* Cuba 1934 strain + mosaic virus, and it was one of the strains used in the serial passages (3, p. 679, footnote 12). "Typical" *X. phaseoli* was masked in the blade of the same trifoliate leaf and isolated therefrom.

(M : P)⁵⁷ and (M : P)⁶¹ = juice from the 57th and 64th serial passages of bean juice containing the mosaic virus + "typical" *X. phaseoli*.

(M + P. var.)⁶⁶ = juice from the 66th serial passage of bean juice containing the mosaic virus the yellow variant of *X. phaseoli* cited above.

P(M + P)³⁴ = an isolate of *X. phaseoli* from the 34th serial passage of the two infective agents. Used for dissociation studies (4).

EXPERIMENT I

Inoculations with (M + P)⁵⁷

The inoculum consisted of crushed tops of plants from the 57th serial passage of bean juice containing both infective agents. One hundred per cent of these plants had severe mosaic but no signs of bacterial infection at the time their tops were used as inoculum, *viz.*, about 3 weeks after inoculation. Mild bacterial stem infection was demonstrated later by microscopic examination.

This experiment was comprised of 8 sets of five 4-inch pots containing

3 Stringless Green Refugee seedlings each, 6 sets with sterilized washed quartz sand, and 2 with sterilized composted soil. Before the unfolding of the first trifoliate leaf, 4 sets (60 plants) were inoculated on the primary leaves. The remaining 4 sets were held as checks. Two days later (March 5) the feeding of the sand cultures with 3 nutrient solutions was begun. These were supplied at the rate of 100 ml. nutrient solution per pot 6 times a week and the 6 sets so fed, including inoculated plants and checks, were designated as "low," "medium," and "high N," respectively.



FIG. 1. A. Stringless Green Refugee bean seedlings inoculated with the crushed tops of plants from the 57th serial passage of bean juice containing *Xanthomonas phaseoli* and the common bean mosaic virus, viz., (M + P)⁵⁷. Experiment I. Left to right: Sand cultures watered with nutrient solutions containing high N (2000 p. p. m.), medium N (200 p. p. m.), and low N (9.8 p. p. m.), and controls in composted soil. Note striking *Xanthomonas phaseoli* infection in medium N. Photographed two weeks after beginning experiment. (Compare noninoculated checks, Figure 1, B.) B. Noninoculated checks, watered with nutrient solutions as in A.

The most interesting thing about these inoculations with (M + P)⁵⁷ was the spectacular outbreak in the medium N set of *Xanthomonas phaseoli* which had been masked in the inoculum. There were very conspicuous typical lesions on the rubbed primary leaves of 100 per cent of the plants (Fig. 1, A). In the high and low N sets, on the other hand, bacterial infection was confined to scarcely discernible water-soaked spots along the veins. In the composted soil set, bacterial infection was somewhat more distinct but by no means conspicuous. Isolates from the 3 nitrogen sets were tested for pathogenicity in experiment III.

Virus infection, manifested by curled trifoliate leaves and much dwarfing, was most striking in the medium N and composted soil sets where there was 100 per cent severe mosaic in 12 days. It was somewhat more severe in the composted soil set. There was also 100 per cent virus infection in the high and low N sets, but it was much less striking.

Expressed juice from each set was tested for pathogenicity of the two infective agents therein (See experiment II).

The effect of the different nutrient solutions on plant development was clearly visible in the noninoculated checks in 5 days after the feeding began. The medium N set was normal in color and size, closely resembling the plants in composted soil. The high N set was dark green and dwarfed, and the low N set was a decidedly yellow green, spindling, and, on this date, the tallest of the three sets of sand cultures. The medium N set outgrew it later (Fig. 1, B). The check plants remained free from infection. None of the inoculated plants ever blossomed.

EXPERIMENT II

Tests of Virulence of the Expressed Juice from Plants Inoculated with (M + P)⁵⁷ and Receiving Varying Amounts of Nitrogen

To test the infectivity of the juice of the inoculated plants in the 3 sand culture sets in experiment I after 3 weeks of feeding with varying amounts of nitrogen, rub-inoculations were made on the primary leaves of 10-day-old Stringless Green Refugee seedlings and, for comparison, on the mosaic-resistant U. S. No. 5 Refugee. A set of each variety was also inoculated with juice from the inoculated plants grown in sterilized composted soil. There were 20 plants in each unit, growing in 6-inch pots of composted soil.

The results indicated that there had been an inhibiting effect of the high and low nitrogen on the bacterium, since, with a single exception,² no visible bacterial infection occurred on either bean variety inoculated with juice from plants in the high or low N sets.

On the contrary 100 per cent of the primary leaves of both bean varieties became infected with *Xanthomonas phaseoli* after they were rubbed with the infected juice of plants from the medium N and composted soil sets. There was also much severe secondary infection on the stems of the mosaic-resistant U. S. No. 5 Refugee. Fifteen out of 20 plants were girdled and broken over in two months.

The virus, on the other hand, was not permanently affected by any of the nitrogen concentrations. In 14 days 100 per cent of the Stringless Green Refugee in all sets were mosaiced. This was likewise the case with plants inoculated with juice from the composted soil set. No mosaic appeared on the U. S. No. 5 Refugee.

Neither bacterial nor virus infection appeared on the noninoculated checks of either variety.

² One plant of U. S. No. 5 Refugee rubbed with inoculum from the high N set became infected.

EXPERIMENT III

*Tests of Virulence of Xanthomonas phaseoli Isolates from Plants
Inoculated with (M + P)⁵⁷ and Receiving Varying
Amounts of Nitrogen*

Isolates of *Xanthomonas phaseoli*, "typical" in appearance in poured plates and on steamed potato cylinders,³ were obtained from the high, medium, and low nitrogen sets in experiment I about 3 weeks after inoculation. Dissociating colonies with many white sectors were also present in poured plates from the low N set.

Comparative inoculations were made on 60 rapidly growing 12-day-old Stringless Green Refugee seedlings in sterilized composted soil using steamed potato cultures from single "typical" colonies as inoculum.

The isolate from the medium N set was by far the most virulent and infection progressed so rapidly that 19 out of 21 plants inoculated were girdled and broken over in 4 weeks, and none of the plants fruited. This was much more severe than the bacterial infection produced by the expressed juice from the medium N set.

Unlike the bacteria in the juice-inoculations described in experiment II, the pure cultures of *Xanthomonas phaseoli* from the high and low N sets were also very virulent. In all 3 sets there was 100 per cent excellent infection with a high percentage of girdled, broken-over stems. The greater pathogenicity of these isolates as contrasted with that of the bacteria in expressed juices from the same source is notable and probably due to a number of factors.

The checks remained healthy and produced a fine crop.

EXPERIMENT IV

*Inoculations with (M + P)⁶⁴, Xanthomonas phaseoli, and
Mosaic Virus*

This experiment, begun June 25, differed from experiment I in (a) the use as inoculum of plants from a serial passage showing 100 per cent visible infection with both pathogens—bacterial as well as virus, (b) addition of a second and third set of inoculations with *Xanthomonas phaseoli* and the mosaic virus, respectively, (c) the use of a far larger number of plants.

The inocula used were as follows:

1. (M + P)⁶⁴: Crushed tops of plants from the 64th serial passage of bean juice containing the two associated infective agents; 100 per cent visible infection with both pathogens. The diseased plant material was collected 18 days after the bean seedlings were inoculated.

2. A sterile distilled water suspension of 3-day-old cultures on steamed potato cylinders of *Xanthomonas phaseoli* Z strain, long in stock, virulent, and with no history of association with the mosaic virus.

3. Crushed typical mosaic-infected trifoliolate leaves from plants inocu-

³ Copious S yellow \pm syrupy growth, so filling the water at the base of the cylinder that the tubes could be turned upside down with impunity.

lated 18 days previously with the juice of infected trifoliate leaves selected from diseased plants in serial passages of mosaic virus-containing juice.

In the sand-grown sets, each unit (*Xanthomonas phaseoli*, low nitrogen, e.g.) contained 100 plants. In the composted soil series, because of a shortage of seed, this number was reduced to 76 in the inoculated set and to 64 in the check set. A total of 1492 Stringless Green Refugee bean seedlings were involved.

The groups treated with different inocula were kept some distance apart. The noninoculated checks on seed infection were isolated.

Four plants were grown per 4-inch pot. The nutrient solutions and schedule of applications used were the same as in experiment I.

(M + P)⁶⁴ Inoculations

Bacterial infection: In the series inoculated with (M + P)⁶⁴, the most severe initial bacterial infection was in the composted soil; the medium N series was a close second. Infection by *Xanthomonas phaseoli* was excellent in the high N set but developed much more slowly. There was ultimately 100 per cent bacterial infection of the primary leaves in all four sets. In those plants suffering from nitrogen starvation (9.8 p. p. m. N), the initial lesions increased but little in size and soon dried up. However, the bacteria reached the stems of at least 65 per cent of the plants, as determined by hand lens examination.

Virus infection: There was severe development of mosaic, with much dwarfing and many curled trifoliate leaves, in both the medium N set and the composted soil, progressing a little more rapidly in the former but reaching 100 per cent in both in 14 days. There was also 100 per cent mosaic in the low N set but it had developed much more slowly. In the high N set very few plants showed signs of mosaic infection other than dwarfing.

The (M + P)⁶⁴ group in experiment IV showed, in 11 days, much more dwarfing (all sets) than did the plants inoculated with mosaic virus alone.

The plants at times appeared to be overwatered by the 100-ml. nutrient solution applied 6 times a week. This, as might be expected, was most marked in the high N set where the plants, in addition to being stunted by the inoculation, were dwarfed by the excess nitrogen.

Xanthomonas phaseoli Z strain inoculations

By far the most severe bacterial infection was obtained in the medium N set. The difference between this and all other sets in the *X. phaseoli* group was conspicuous. There was 100 per cent heavy infection of the inoculated primary leaves and 68 per cent stem infection in the medium N set.

In the low N set, good but scattered lesions were on the primary leaves, and 62 per cent visible secondary infection on the stems. Yellow bacterial ooze was produced in 32 per cent of the infections by the 29th day.

In the high N set, a high percentage of the rubbed primary leaves had a very slowly developing infection, although at the end of two weeks these

plants had the fewest bacterial lesions of any set in the *X. phaseoli* group. There was never any visible secondary infection on the stems which were under observation for 38 days.

In composted soil there was only scattered infection on the primary leaves but 42 per cent visible stem infection in 30 days.

Mosaic virus inoculations

Virus infection in this group was very poor as a whole. The best, a little mild mosaic with some curling of trifoliolate leaves, appeared in the sand with medium N and the composted soil sets. There was some dwarfing in the low N set, which may have been due to the virus, but no other mosaic symptoms appeared. There was little if any virus infection in the high N set. These results were puzzling and might have been laid to the failure to use carborundum had it not been for the fact that in the serial passages of juice containing the virus alone, infection had never fallen below 80 per cent and in 7 of the 10 passages completed at this time⁴ had reached 100 per cent good but not severe mosaic infection without the use of carborundum.

The most striking thing about this virus group was the outbreak of typical *Xanthomonas phaseoli* infection in 73 per cent of the plants in the medium N set though no bacterial infection had been observed in the plants in the mosaic-virus serial passages from which the inoculum was obtained. A little bacterial infection also occurred in the high N set but none in the low N or soil sets. Apparently *X. phaseoli* was masked in the inoculum and, as in experiment I, it had been stimulated by the 200 p. p. m. N in the medium N set.

Checks

There was no sign of virus or bacterial infection in any of the 300 non-inoculated check plants growing in sand or in the 64 in composted soil. The optimum growth was in the medium N set.

Overwatering

It was apparent that 100 ml. of nutrient solution 6 times a week constituted overwatering for the dwarfed plants, particularly those in the high N sets of all groups including the noninoculated checks. On the other hand it was sometimes not quite enough for the groups in which the plants made a normal amount of growth, notably the *Xanthomonas phaseoli*, virus, and check groups in the medium N set.

Beginning with the 21st day the high N sets in all groups were fed every other day and water was given here and there as needed.

EXPERIMENT V

Inoculations with (M + P. var.)⁶⁶, Xanthomonas phaseoli and Mosaic Virus

As previous experience had shown that the dwarfed sets were frequently

⁴ The 10th serial passage had been made the day following the inoculations in this virus group.

overwatered, experiment IV was repeated the first of October, using 6-inch pots⁵ with 4 plants each and adding water here and there as needed. As a result none of the sets showed ill effects of overwatering. Sixty plants comprised each of the sand units and 48 plants the composted soil units. The feeding began October 5, the day after inoculation.

Aside from the fact that the virus inoculum used in this experiment was more virulent, the results previously obtained were confirmed using another set of inocula as follows:

1. (M + P. var.)⁶⁶: Tops of plants from the 66th serial passage of juice containing the mosaic virus and the yellow variant of *X. phaseoli* described in an earlier paper (3). One hundred per cent of the plants of the 66th serial passage had excellent mosaic and scattering mild *X. phaseoli* infection. The material was collected three weeks after inoculation.

2. *Xanthomonas phaseoli*: A sterile distilled water suspension of cultures on steamed potato cylinders of the Wyoming 1940 strain, a very virulent isolate recently obtained from a bean pod and having no history of association with the mosaic virus.

3. *Virus inoculum*: From serial passages of juice containing mosaic virus. They had been started 8 months earlier by the use of seed-infected plants as inoculum. The plants had been inoculated 4 weeks previously.

Crop

Unlike the previous experiments, experiment V was continued long enough to form an estimate of the crop, that is, until the plants were 7½ weeks old. In all sets the crop was much reduced by infection, most in the (M + P. var.)⁶⁶ group which did not even blossom, next in the virus group, least by *X. phaseoli*.

Only the checks were in condition to mature all the pods on the plants at the time the count was made. They produced about 1.4 times as many in composted soil as in the medium N set, and very few in low and high N, least in the latter.

DISCUSSION

Although various investigators have reported on the effect of nitrogen on the activity of plant pathogens, none, to the authors' knowledge, have worked on its effect on associated infective agents. The present investigations were carried on from that point of view.

In the studies here reported, the results may have been somewhat different had not root nodules formed notwithstanding the sterilization of the washed quartz sand in which the seedlings fed with nutrient solutions were grown. It is quite conceivable that 200 p. p. m. N would not have been sufficient for 2 pathogens in addition to the host without the extra N supplied by the root nodules. Scattered small nodules and a few large ones were formed in plants in this medium N set. In the high N (2000 p. p. m.) set, there were scattered small nodules and in the low N (9.8 p. p. m.) set, scat-

⁵ Six-inch pots contain 3½ times as much soil as 4-inch pots.

tered large ones. L. T. Leonard states (oral communication) that under ordinary greenhouse conditions it is almost impossible to exclude the nodule bacteria when growing many species of legumes.

A comparison of the bacterial infection produced by inoculations with (A) the expressed juice from plants of the 3 nitrogen sets of (M + P)⁵⁷ inoculations in experiment I (See experiment II), and (B) steamed potato cultures of isolates from the same nitrogen sets (See experiment III) is illuminating. The lesser pathogenicity of the bacterium in the expressed juice inocula is believed to be due (a) to its long *in vivo* existence in the much stunted plants of the serial passages in association with the virus, in which circumstances a tendency to dissociation into less virulent and avirulent variants has been demonstrated (4) and (b), in the case of the bacterium in the juice from the high and low N sets, to the additional inhibiting influence of excess and deficient nitrogen. On the other hand the greater pathogenicity of the pure cultures of the isolates is in all probability due to the preliminary building up of their virulence on a favorable culture medium (steamed potato cylinders) which favors the virulent S yellow form and tends to repress the mildly pathogenic and avirulent variants.

Unfortunately none of the available virus inoculum was absolutely free from the suspicion of the presence of masked *Xanthomonas phaseoli*. Just before making the last set of inoculations in the virus group (experiment V), bacterial infection broke out for the first time in the source of inoculum, *viz.*, the mosaic virus serial passages carried on for 8 months. It was such an outbreak of *X. phaseoli* in Zaumeyer's tests of hybrids for mosaic resistance that had led to the first association studies by the senior author (3).

There was no mosaic-infected seed at hand from which a fresh start towards obtaining virus inoculum could be made. Hence the inoculum for the virus group cannot be considered assuredly pure virus comparable to pure cultures of *Xanthomonas phaseoli*. As for the virus infection in the virus group, it is possible that better results would have been obtained with the use of carborundum although 100 per cent good but not severe mosaic infection had occurred without its use in 10 out of 14 serial passages of the virus completed up to this time. Furthermore, in none of the 14 passages had infection fallen below 80 per cent.

SUMMARY

The effect of varying amounts of nitrogen, 9.8 p. p. m., 200 p. p. m., and 2000 p. p. m., was tested on Stringless Green Refugee bean seedlings grown in sand and inoculated with (a) juice from serial passages of bean juice containing *Xanthomonas phaseoli* and the common bean mosaic virus, (b) pure, single colony cultures of *X. phaseoli*, and (c) juice from serial passages of bean juice containing the common bean mosaic virus. Comparative inoculations were made on plants grown in sterilized composted soil and noninoculated plants, grown in each substratum, were held as checks on seed infection.

Of the three concentrations of nitrogen tested, medium N (200 p. p. m.)

produced the most intense development of both virus and bacterium in every case. This was also optimum for growth of the host.

Pathogenicity tests of juice from all sets of plants inoculated with (M + P)⁵⁷ in experiment I showed that the virus had not been permanently inhibited by any of the three N-concentrations, inasmuch as the juice from all sets produced 100 per cent good mosaic when tested on plants grown in composted soil. On the other hand there had been marked inhibition of the bacterium by both low N (9.8 p. p. m.) and high N (2000 p. p. m.).

When, however, isolates of the bacterium from the same three nitrogen sets were first grown on a favorable culture medium (steamed potato cylinders) and then used as inoculum, all were very virulent although the isolate from the medium N (200 p. p. m.) was notably more so.

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TYPES OF DEFENSIVE REACTIONS IN PLANTS

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By a defensive reaction we understand a vital process initiated in a host by a pathogenic agent and directed more or less specifically against that agent itself. In a book recently issued, the writer (6) has endeavored to discuss in detail the various types of defensive reactions in plants. It is the purpose of this article to represent certain points of view as set forth in that treatise.

The defensive reactions in plants may be broadly classified as follows:

1. According to the object against which they are primarily directed;
2. According to the life history of the infected plant;
3. According to the intensity of the reaction.

The object of the defensive reactions. According to their object the defensive reactions may in turn be grouped in three subdivisions, viz:

- a. The anti-infectional reactions, which are directed against the parasite itself and endeavor to weaken or annihilate it;

- b. The antitoxic reactions, which are not primarily directed against the parasite, but against its metabolic products, especially its toxins, and, furthermore, against the necrotic products of the affected cells and tissues, which they tend to render harmless;

- c. The induced tolerance, which (contrary to the previously given definition) is directed neither against the parasite nor against its metabolic products; it acts rather against the sensitiveness of the host plant itself to these pathogenic agents. The host plant undergoes a desensibilization by virtue of which it no longer reacts to the pathogenic agent. The plant tolerates the latter without manifest symptoms and, although infected, does not become diseased.

The life history of the infected plant. The capacity for reacting defensively is either spontaneous (autonomous) or acquired (induced). The first is hereditary, endowed by nature; the second remains merely potential, latent, until it is activated by a primary infection; by withstanding this the plant's readiness to resist is sensitized so that only then does it develop its full defensive capacity.

The intensity of the defensive reactions. Reactions are either normergic or hyperergic. In normergic reactions (which need not necessarily be merely defensive) cause and effect bear a certain proportionate ("normal") relation to each other. When parasite and host bear such a normal relationship, it is termed eusymbiotic; although each partner is in conflict with the other, they still continue together in a kind of parasitic community, which leads to disease. The hyperergic reactions represent the other extreme. The ratio between cause and effect becomes disproportionate. A host-parasite relationship of this kind is termed parabiotic; the two part-

ners do not tolerate each other; they react transversely to each other, with the result that within a few hours necrotic alterations in the infected cells, or in the adjoining noninfected cells, manifest themselves and rapidly cause death.

In accordance with these viewpoints it was attempted to classify in table 1 the different types of defensive reactions so far as they have been exactly determined in plants.

I. The plasmatic anti-infectious reactions. These represent the normergic type of anti-infectious defense, most easily traceable in cases of chronic infections, when parasite and host are in contact over protracted periods. Similar reactions doubtless occur in the course of acute disease, but, being more rapid and irregular, are more difficult to observe and to interpret. They operate along three lines.

TABLE 1.—*The types of defensive reactions in plants. After Gäumann (6)*

Object of the reaction	Historical development	Intensity	Type of reaction
The pathogen itself (anti-infectious reactions)	Autonomous	Normergic	I. Plasmatic anti-infectious reactions
		Hyperergic	II. Necrotic reactions
	Induced	Normergic	III. Premunity
The metabolic products of the pathogen (anti-toxic reactions)	Autonomous	Normergic	IV. Histogenic demarcations
		Hyperergic	V. Gummous demarcations
The innate sensitiveness of the host (induced tolerance)	Induced	Normergic	VI. Desensibilization

a. They alter and weaken the invading parasite. For example, *Bacterium radicola* Beij. destroys the root cells of weakened Leguminosae. In vital plants, however, the bacterium is altered under the influence of the defensive reactions: the host cells remain alive and retain ability to divide despite being flooded by the bacteria (17). To take a mycological example: when lateral hyphae of intercellular fungi enter the host cells, the hyphae generally stop growing and become knotty and deformed. Since they dissolve and absorb the contents of the host cells, their peculiar form is regarded as fungoid ("haustoria"). This assumption is not unreservedly right; when the same fungi are grown on dead tissues of the same hosts, they form no such "haustoria" in the host cells, although they extract their nourishment from the latter. Hence, the haustoria are not a growth-form determined by the fungus, but are reaction-products between the defensive reactions of the host cells and the counter-reaction of the intruding hyphae.

b. They localize the intruding pathogen. Only a few infectious diseases of plants are systemic; most of them are localized (*e.g.*, leaf-diseases).

We may assume that the reason lies in the functional defense of the host cells, which causes an exhaustion of the invading agent and finishes by stopping and localizing the infection. When the same leaves are first killed by freezing, the same parasites (e.g., *Cercospora beticola*, *Venturia inaequalis*, *Diplocarpon rosae*) are able to grow right through them without restraint.

c. They digest and eliminate the intracellular parasites. Thus, haustoria are in time corroded and live for a relatively shorter period than the intercellular hyphae. The intracellular bacteria of the root-nodules (Fig. 1) and the intracellular hyphae of mycorrhizae (Fig. 2) furnish further examples of these digestive processes.



FIG. 1. Digestion of *Bacterium radicicola* Beij. in the root-nodules of *Lupinus albus* L. 1500 x. After Schaede (14).



FIG. 2. Digestion of mycorrhiza in the roots of *Platanthera chlorantha* Cust. Left, a cell rich in content, showing incipient digestion. Right, a cell poor in content, showing an advanced stage of digestion. 160 x. After Burgeff (4).

These three normergic principles (weakening, localizing, and eliminating the parasite) are based on biochemical reactions; they are borne by agglutinins, lysins, etc., as is proved by appropriately selected examples. Compared with the anti-infectious reactions of the human body their efficiency is low. Not only do they generally fail to prevent the infection, but they also are mostly unable to eliminate the invading parasite; hence, they do not lead to an autosterilization, to healing of the infected tissues; these remain, as a rule, affected for the rest of their life. Accordingly plants in general do not recover from their infectious diseases.

The cause of this inefficiency doubtless is to be attributed to the extreme simplicity of the mechanism of the plasmatic defensive reactions. In the

human and animal body, invading germs are combatted not only by the biochemical action of agglutinins, lysins, etc., but mainly by special resistance cells, viz., by the mobile leuco-phagocytes and by the stationary reticulo-endothelial system. Above all, the mobile defensive system enables the host to eliminate the pathogen within a short time, by assembling at the point of danger hundreds of thousands of fighting-cells, which eliminate, by fighting one cell against another, the invading germs. Such mobile fighting cells are unknown to the plant; hence it is unable to mobilize at the point of infection the full resistance-potential of entire tissues or organs. Each infected cell-group must resist by itself, independently and without help from other cells. Hence, it is not surprising that the normergic plasmatic defense system in plants meets with such a low degree of success, merely localizing, but not healing, the infections.

II. The necrotic reactions. These represent the hyperergic type of reactions and are caused by a hypersensitiveness of the host cells to the emanations of the parasite. Their consequence for the parasite-host system are diametrically opposed according to the biological type of the parasite, which may be either necrotrophic or biotrophic.

Necrotrophic parasites (e.g., *Botrytis cinerea*, *Valsa*, *Corticium vagum*) are able to feed on dead substances of the host. If, therefore, the host cells die at their approach, the further development of the parasite is facilitated.

Biotrophic parasites (e.g., *Synchytrium endobioticum*, mildews, rusts) on the contrary, can only nourish themselves from the living substance of the host. Hence if their intrusion is met by hyperergic reactions of the host cells and if the latter perish, the further development of the parasite is rendered impossible, a sort of scorched earth strategy in nature. Accordingly, necrotic reactions are not actually defensive reactions, but rather the evidence of the highest susceptibility or hypersensitiveness. But since the invading parasite is destroyed simultaneously with the death of the host cells, these hyperergic reactions have an anti-infectional effect: hypersensitive individuals are resistant to biotrophic parasites because they are over-susceptible.

The timing of the hyperergic response varies. The epidermal cells of red clover perish at the very first contact with the infectious hyphae of *Erysiphe martii* (Fig. 3, A and B), while in Kanred wheat the symptoms of the hyperergic effects appear only after some days (Fig. 3, C).

III. Premunity. In the reactions so far dealt with, the full readiness and capacity for reaction were innate to the individual (autonomous reactions). Among the numerous reactions which are initiated in the invaded individuals by the pathogen, and which mostly lead to a damaging of the former, there are nevertheless some which occasionally produce the opposite result. Instead of weakening the host, the attacking pathogen stimulates or sensitizes it, activating in it a resistance power which was previously only latent or insufficient (induced defensive reactions).

In the field of induced defensive reactions, however, the premises in phytopathology and in human and veterinary medicine are rather different. In vaccination, it is the recovery from an artificially introduced infection, which leads to an alteration in the reaction-norm of the organism. The pathogen disappears from the body, which however retains an increased resistance power against a later re-infection.

This form of acquired immunity can scarcely be expected in plant life, because plants cannot, as a rule, be entirely freed of infection. They are, however, capable of attaining a different type of immunity, a type known in human medicine (dermatology), but of no great significance there; this is infectionary immunity or premunity, based on the fact that an organism or certain of its tissues, cannot be effectually superinfected by the same or

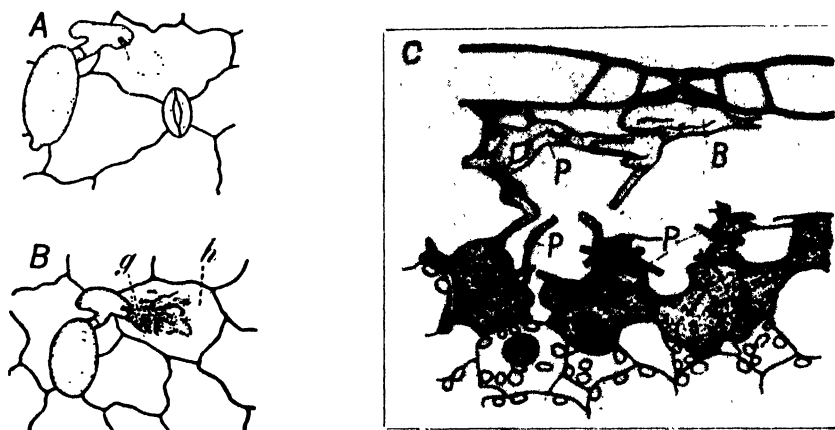


FIG. 3. A and B. Infection of *Trifolium pratense* L. by *Erysiphe martii* Lév. A. A susceptible variety of clover, which only reacts normergically, and in which the parasite can form a haustorium, without causing any apparent disturbance. B. A hypersensitive variety, in which the invaded epidermal cells undergo a sudden necrotic degeneration and perish together with the intruding parasite. *g*, infection hypha; *h*, necrotic host-cell. 385 x. After O. F. Smith (15). C. Hyperergic reaction of Kanred wheat to an infection of *Puccinia graminis tritici* Erikss., biotype 1. B, sub-stomatal vesicle; P, fungus hyphae, partially abortive. Approx. 350 x. After Allen (1).

by a related pathogen, so long as the original infection exists. In acquired immunity, the curing of a primary infection provides protection against a re-infection, whereas, in premunity, a still-existing primary infection provides protection against a super-infection.

Accordingly, premunity, like acquired immunity, is based upon an alteration in the host, effected by pre-infection, through which the host has gained a resistance power which it did not originally possess. In contrast to acquired immunity, the alteration in the host is maintained in the case of premunity only as long as the pre-infection lasts.

Premunity can, according to the field of its action, produce either a local immunity from infection or a local immunity from disease.

A classical example of a local immunity from infection is furnished by Bernard's experiments with orchid mycorrhiza (2). If young orchid em-

bryos be infected by a less aggressive strain of mycorrhiza, the hyphae penetrate at the suspensor region to a depth of several cell layers; their further progress is then checked. If, however, after some days, the pre-infected embryos be subjected to a virulent fungus strain, one so virulent as would normally kill the embryo, this fungus is no longer able to enter the infected region. When, however, in later stages, the embryo produces bristles, the lethal infection can enter through these. The embryo's readiness to resist infection has thus been only locally sensitized. This local premunity is group-specific; if it is initiated by a weak strain of *Rhizoctonia repens* Bern., it is also effective against aggressive strains of *Rhizoctonia mucoroides* Bern. and of *R. lanuginosa* Bern.

Premunizing experiments made by Salaman (11, 12) with the X-virus may be mentioned as an example of an induced local immunity against disease. A number of tobacco plants were preinfected with the weakest G-strain, the symptoms of which were so mild as to cause only the suspicion of a mottling. The plants were then superinfected by the virulent S-strain in consecutive groups, at 24-hour intervals. In the superinfections of the first 4 days the S-strain penetrated and the plants became diseased according to the S-type. The group superinfected on the fifth day evinced a partial resistance to the S-strain-disease, while the group treated on the eighth day no longer showed any symptoms of S-disease; the plants in this group reacted only to the G-strain: in them, the S-virus could not develop beyond the point of infection.

Thus, previously infected individuals remain susceptible to superinfection. After a period of latency (4 days), however, they become capable of arresting the general spreading of the superinfected virus; they localize it, and therefore become resistant to disease, just as in the case of human beings who have suffered from diphtheria. The induced protection is specific in such cases and is not effective against Y- and ring-spot viruses. In other cases, however, the premunity is group-specific and, furthermore, its effect can reach a depth of 7 cell layers beyond the pre-infected zone, thus leading to a local tissue-immunity (5, 7, 18).

IV. Histogenic demarcations. The defensive reactions so far dealt with are exclusively of anti-infectional type, being directed against the parasite. As, however, they generally succeed in merely weakening and localizing, but not eliminating it, a chronic disease-focus results. Consequently, the host organism has the new task of seeking to protect itself against the pathogenic metabolic products emanating from the focus; especially against the toxins of the parasite and the necrotic products of its own invaded cells. This protection is effected by means of the antitoxic reactions; their aim is not to prevent infection, but to prevent disease.

In the human system such antitoxic protection is provided biochemically by means of specific antitoxins, which neutralize the pathogenic agent (destroying it functionally), and histologically by means of demarcating tissues.

In plant pathology antitoxins have not yet been found, but numerous demarcation reactions are known; histogenic demarcations (Fig. 4) may be mentioned as a normergic example, and gummous demarcations as an hyperergic example.

To judge by such investigations as have been made on histogenic demarcations, they possess chiefly antitoxic, not anti-infectional properties; therefore the parasite is sometimes able to penetrate them. The reaction chain is not initiated by the parasite itself, but by the necrosis caused by it. To this extent, demarcation reactions are nonspecific, being indifferent to the particular source of the necrosis, which may be one of the following: necrogenic products of the lesions, toxins of the parasite, or any other organic or inorganic poisons. Similarly the mechanism of demarcation is

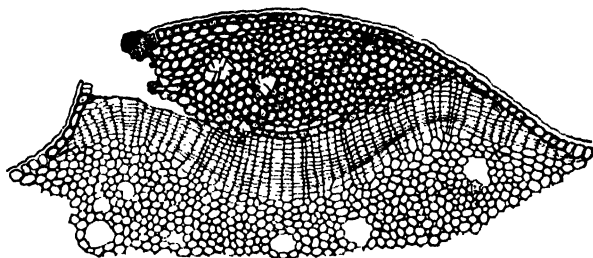


FIG. 4. Focus of *Clasterosporium carpophilum* (Lév.) Aderh. on branch of almond, *Prunus amygdalus* Stok., confined by a demarcation tissue. 60 \times . After Samuel (13).

nonspecific; it corresponds to the widespread manifestation that local lesions produce a localizing tissue in plant bodies (deliscence tissue, scar tissue, etc.).

V. Gummous demarcations. An example of the antitoxic effect of a hyperergic gummosis may be found in the silver-leaf disease of plum trees. If a root or the trunk be locally infected by *Stereum purpureum*, toxins of this agent are carried with the sap into the upper regions of the tree, where they cause cellular pathological changes (silver-leaf effect). In severe cases the disease can lead to the death of the tree within a few years.

In certain varieties of plum trees, the capacity of resistance to disease depends upon the readiness of the woody tissues to respond with hyperergic antitoxic reactions. The Victoria plum reacts weakly and therefore falls an easy victim to the disease (3). On the other hand the trunks of Pershore plum respond with an ample exudation of gum, which forms an impassable barricade to the toxins, similar to the reaction zone in beeches (Fig. 5). The regular formation of this antitoxic zone in the beech possibly provides the reason why this species is not liable to silver-leaf disease, notwithstanding its continual contamination by *Stereum purpureum*.

Both plum varieties mentioned are liable to infection; but the Pershore variety is better able to resist disease because it is able to prevent the flooding and poisoning of its tissues by the pathogenic agents emanating from the focus.

VI. Induced tolerance. Even when a plant is unable to localize the pathogenic agent (the parasite or its toxins) and is consequently flooded by these, its condition is not necessarily hopeless. It can, in certain cases, resort to a new form of disease prevention, by simply ignoring the agent. Even though flooded by the pathogen the plant does not react to it, in short, it becomes tolerant (induced tolerance).

Induced tolerance has one feature in common with the antitoxic reactions, in that it shuts out the result of the infection; but, not as the latter does, by neutralizing or localizing the harmful agent, but rather by adopting the opposite extreme of behavior. It effects a desensibilization of its own system, which no longer answers to the pathogenic agent, and there-



FIG. 5. Antitoxic reaction of a beech trunk to artificial infection by *Stereum purpureum*. Approx. half nat. size. After Münch (8).

fore does not develop the disease. Human medicine possesses an indication of such induced tolerance in typhus carriers.

For instance, if the virus of the ring-spot disease be transferred to healthy tobacco plants by means of rubbing the leaves, typical symptoms appear on these leaves within three days, and the virus spreads through all parts of the plant, with the exception of the growing point. However, the symptoms gradually begin to fade out; those in newly formed leaves and lateral branches become increasingly faint and eventually disappear entirely. The leaves on such plants are merely somewhat darker green and more leathery than on virus-free specimens (9, 10). In the meantime the leaves originally infected have dropped off; the plants have "recovered"; they are clinically cured, but continue to be carriers of the virus, which is still present, unweakened and fully pathogenic to uninfected plants. Only the symptoms have disappeared.

This clinical healing is apparently based on three components:

1. Actual anti-infectious reactions (formation of anti-virus bodies). As a result of these, the virus protein content in recovered leaves is, in general, only 10-20 per cent of that in the originally diseased leaves.

2. A blocking of the remaining 10-20 per cent of the virus proteins. These are in no way blunted, but fully pathogenic to other plants; only in the "healed" plants are they unable to destroy the chloroplasts and to cause necrotic degeneration.

3. A desensitizing of the host organism, *i.e.*, a conversion to tolerance, by virtue of which the host is able to carry the remaining virus (which assimilates a considerable part of the host's native protein) without manifesting symptoms.

When clinically healed carriers are strongly superinfected with the ring-spot virus, they evince no further symptoms; they have thus become tolerant towards even a severe superinfection. This induced tolerance is group-specific against all types of ring-spot virus; the latter can develop undisturbed in the "cured" plants, while retaining full pathogenicity for other plants; only the "recovered" plants ignore, and do not react to them.

If a healthy shoot be grafted on to the stem of a "recovered" tobacco plant, it takes up the virus, but not the acquired tolerance, so that it evinces incipient symptoms of ring-spot which, however, later fade out.

In curly-top of sugar beet however, inoculated to tobacco, not only the virus, but also the induced tolerance entity emigrates to the grafted shoots (19). The "recovered" partner thus induces in the healthy partner another reaction-norm against the virus; a similar induction is found in the organizer-effect in animal transplantations (16).

These six main types of defensive reactions probably do not exhaust the possibilities of active resistance in plants. The aim of this article is merely to describe them shortly with a consistent terminology.

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TEN ADDITIONAL LEAFHOPPER VECTORS OF THE VIRUS CAUSING PIERCE'S DISEASE OF GRAPES

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INTRODUCTION

Three species of leafhoppers, *Draculacephala minerva* Ball, *Carneocephala fulgida* Nott., and *Neokolla circellata* (Baker), were stated by Hewitt, Frazier, Jacob, and Freitag (4) in 1942 to have the ability to transmit the virus causing Pierce's disease of grapes. Frazier (3) reported these three and, in addition, the following six species as vectors in 1944; *Carneocephala triguttata* Nott., *Helochara delta* Oman, *Neokolla gothica* (Sign.), *N. confluens* (Uhler), *N. hieroglyphica* (Say) and *Cuerna occidentalis* Oman and Beamer.

Hewitt, Houston, Frazier, and Freitag (5) later published experimental data substantiating the fact that *Draculacephala minerva*, *Carneocephala fulgida*, *Neokolla circellata* and *Helochara delta* can transmit the virus, and showed that alfalfa dwarf disease and Pierce's disease of grape are caused by the same virus.

The present paper presents experimental data supporting the fact that the remaining 5 previously reported species, namely, *Carneocephala triguttata*, *Neokolla gothica*, *N. confluens*, *N. hieroglyphica*, and *Cuerna occidentalis* are vectors of the virus, and contains evidence to show that an additional 5 species of leafhoppers, not previously reported, are also able to transmit the virus. They are *Pagaronia triunata* Ball, *P. 13-punctata* Ball, *P. furcata* Oman, *P. confusa* Oman, and *Friscanus friscanus* (Ball).³

METHODS

All work herein reported was carried out at Berkeley under glasshouse conditions. Naturally and experimentally infected alfalfa and grape plants were used as sources of virus for the leafhoppers. Healthy California Common variety alfalfa plants and grapevines of the varieties Emperor, Thompson Seedless, Ribier, and Palomino propagated from indexed cuttings were used as test plants. The numbers of tests made of the different species of leafhoppers were determined in large measure by their availability. The numbers of insects used per test were usually from 5 to 15, but the great majority of tests was made with 10. It was not found possible to effectively rear sufficient populations of any of the species for experimental work, so it was necessary to collect all insects in the field. Because of limited distri-

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³ Credit is due to Dr. R. H. Beamer for the determinations of *Carneocephala triguttata* and *Cuerna occidentalis*; to Dr. D. M. Delong for determining *Neokolla gothica*, *N. confluens*, *N. hieroglyphica*, and *Friscanus friscanus*; and to Dr. R. I. Sailer for determining *Pagaronia triunata*, *P. furcata* and *P. 13-punctata*.

bution and relative scarcity of some species often only small numbers could thus be obtained. With the genera *Pagaronia* and *Friscanus*, grape and alfalfa proved to be rather unfavorable hosts inasmuch as a relatively high mortality resulted, and this again limited the number of tests.

TRANSMISSION OF VIRUS

The results of transmission trials carried out with 10 species of leafhoppers are in table 1. Under the conditions of these experiments a considerable range was evidenced among the various species in their relative ability to transmit the virus from diseased to healthy alfalfa and grape plants. The most efficient group of vectors included the species of the genera *Carnecephala*, *Neokolla*, and *Cuerna* which infected from 42 to 78 per cent of the total number of plants inoculated. Grape or alfalfa were relatively favorable host plants for the species belonging to these 3 genera,

TABLE 1.—Transmission by 10 species of leafhoppers of virus from diseased to healthy grape and alfalfa plants

Species of leafhoppers	From diseased grape		From diseased alfalfa		Percentage of total infected
	To grape	To alfalfa	To grape	To alfalfa	
<i>Carnecephala triguttata</i>	3/4*	0/1	3/7	4/12	42
<i>Neokolla gothica</i>	13/16	3/5	8/15	4/21	49
<i>N. conflucus</i>	7/7	1/3	4/6	2/2	78
<i>N. microglyphica</i>	2/2	0/0	2/4	1/4	50
<i>Cuerna occidentalis</i>	9/10	2/3	3/3	2/4	76
<i>Pagaronia triunata</i>	0/7	0/3	0/3	3/10	13
<i>P. 13-punctata</i>	0/0	0/0	0/0	1/3	33
<i>P. furcata</i>	9/35	2/13	5/25	5/39	19
<i>P. confusa</i>	2/23	1/16	3/14	4/16	14
<i>Friscanus friscanus</i>	0/0	0/0	3/3	3/25	21

* The numerator indicates the number of plants infected; the denominator indicates the number of plants inoculated.

as judged by the longevity of the insects when caged on them. The species of the genera *Pagaronia* and *Friscanus*, however, survived relatively poorly on grape or alfalfa, and with these species the percentage of transmission of virus, as shown in table 1, was usually low, ranging from 13 to 33. In the cases of *P. triunata* which yielded but 3 infections of 23 tests and *P. 13-punctata* which was tested only 3 times and caused only 1 infection it is evident that, if considered separately, the number of cases of infection is too low ordinarily to be acceptable as proof of a species' ability to transmit a virus. These 2 species are listed in this instance, however, because 2 other species in the same genus yielded a comparably low but consistent number of infections over a much larger series of tests and it seems safe to assume that the same would hold true should *P. triunata* and *P. 13-punctata* be subjected to further testing.

None of the 10 species listed in table 1 appears to be of any importance under field conditions in the spread of the virus to either alfalfa or grapevines since they are only rarely found in vineyards or alfalfa fields.

PHYLOGENETIC RELATIONSHIP OF PIERCE'S DISEASE VECTORS

Fourteen species of leafhoppers in 7 genera, listed earlier in this paper, have been shown to be capable of transmitting the virus of Pierce's disease. They are all contained in the leafhopper (Homoptera-Cicadellidae) subfamily Tettigoniellinae (2) (= Cicadellinae (6) = Amblycephalinae (1)), members of which are commonly known as sharpshooters.

Early in the search for a vector of the virus a valuable clue was provided in a vineyard in which insect-electrocuting light traps were being operated as described in a previous publication (5). Under certain of the traps very definite accumulations of diseased vines had occurred (5, fig. 1) and under these same traps marked concentrations of populations of *Draculacephala minerva* and *Carneoccephala fulgida* were noted. Other factors also served to strengthen the suspicion that either or both insects might be the virus carrier. Subsequent tests proved both to be vectors.

Neokolla circellata became strongly suspected of being a vector because of its abundance on wild and cultivated grapevines in localities where Pierce's disease was prevalent and neither of the known vectors, *Draculacephala minerva* or *Carneoccephala fulgida*, was present in sufficient numbers to satisfactorily account for the amount of disease.

When *Neokolla circellata* was demonstrated to be a vector of Pierce's disease virus it became the third species belonging to the subfamily Tettigoniellinae known to have the ability to transmit the virus, and the other species of the subfamily were then suspected as possible vectors. Consequently an effort was made to test as many species as conveniently possible in order to explore the possibility that the ability to transmit the virus might be common to all species of the subfamily. That the ability to transmit the virus can definitely be correlated with a phylogenetic relationship of the vectors within subfamily limits is evidenced by the fact that every species of the subfamily Tettigoniellinae thus far tested has the ability to transmit the virus. During the course of the experiments more than 50 species of leafhoppers contained in subfamilies other than Tettigoniellinae were tested and not one proved to be a vector.

It is not implied that the ability to transmit the virus of Pierce's disease is necessarily specific or limited only to species within the Tettigoniellinae. It is quite possible that species in other subfamilies or families may have that ability. Nor is it implied that every species of the Tettigoniellinae must necessarily be a vector, although the evidence strongly suggests that the ability to transmit the virus is a group characteristic and each species should be suspected of the ability until proven otherwise.

SUMMARY

Ten additional species of leafhoppers are demonstrated to be vectors of the virus of Pierce's disease of grapes. These are *Carneoccephala triguttata*, *Neokolla gothica*, *N. confluens*, *N. heiroglyphica*, *Cuerna occidentalis*, *Pugaronia triunata*, *P. 13-punctata*, *P. furcata*, *P. confusa*, and *Friscanus friscanus*.

None of the 10 species appears to be of any importance under field conditions in the spread of the virus to alfalfa or grapevines.

All 14 vectors of Pierce's-disease virus thus far demonstrated are contained in the leafhopper subfamily Tettigoniellinae of which every species thus far tested has transmitted the virus. More than 50 species of leafhoppers contained in other subfamilies were tested and not one proved to be a vector. The evidence indicates that the ability to transmit the virus can be correlated with a phylogenetic relationship of the vectors within the Tettigoniellinae.

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EFFECT OF SOIL TEMPERATURE ON THE DEVELOPMENT OF RHIZOCTONIA ROOT CANCER OF ALFALFA¹

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It is well known that soil temperature influences the development of certain plant diseases caused by *Rhizoctonia solani* Kühn. In some cases (1, 2, 3, and 8) the disease develops best at high soil temperatures; whereas, in other cases (4, 5, 6) it develops best at low soil temperatures. In a previous publication (7) it was noted that *Rhizoctonia* root canker of alfalfa is seasonal in its development and is closely correlated with warm soil temperatures. Cankers develop most abundantly during June, July, August, and September when soil temperatures, at 3 inches below the surface, are above 20° C., but no cankers develop during the winter months when soil temperatures, at this same depth, may be as low as 5° to 10° C. This seasonal development of the disease suggests that soil temperature is an important factor influencing its development.

This paper reports results from experiments to determine the influence of temperature on the growth of *Rhizoctonia solani* and the effect of soil temperature on the development of *Rhizoctonia* root canker of alfalfa.

EFFECT OF TEMPERATURE ON GROWTH OF THE ORGANISM

The effect of temperature on growth of the organism was determined by growing the organism, in triplicate, in Petri dishes on potato-dextrose

TABLE 1.—Number of cankers per main tap root of alfalfa plants inoculated with isolate 102 of *Rhizoctonia solani* from alfalfa and grown at different soil temperatures for 50 days

No. of roots	No. of cankers per root after 50 days at soil temperatures of:					
	15° C.	20° C.	25° C.	30° C.	35° C.	40° C.
25	0	13.3	21.3	29.4	6.1	0

agar at constant temperatures of 5°, 10°, 15°, 20°, 25°, 30°, 35°, and 40° C.³ A small amount of mycelium was placed at the center of each dish and the diameters of the colonies determined at the end of 3 days (Fig. 1). The organism grew most rapidly at 25° and 30° C. No growth occurred at 5°, 10°, and 40° C. during the 3-day period. In another experiment, a small amount of growth occurred at 10° C. after 11 days.

¹ Cooperative investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Nevada Agricultural Experiment Station.

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³ Relation of temperature to growth on potato-dextrose agar was determined by H. W. Johnson, Senior Pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

EFFECT OF SOIL TEMPERATURE ON DISEASE DEVELOPMENT

For determining the effect of soil temperature on disease development, 4-months-old alfalfa plants growing in galvanized iron cans (25 plants per can) were inoculated with culture 102 of *Rhizoctonia solani* and maintained at soil temperatures of 15°, 20°, 25°, 30°, 35°, and 40° C. for 50 days. Soil temperatures were maintained by thermostatically controlled water baths. Inoculation technique was the same as described elsewhere (7). At the end of 50 days the roots were removed from the soil and the number of cankers on the main tap root was determined (Table 1). There were no root cankers at soil temperatures of 15° and 40° C. and an average of 29.4

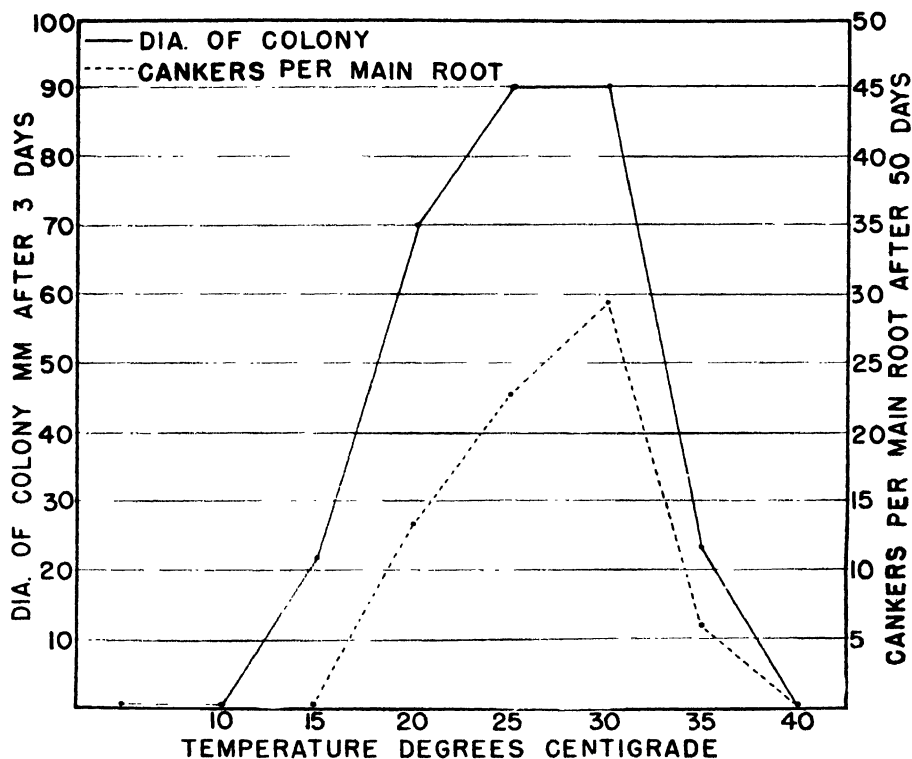


FIG. 1. Effect of temperature on growth of *Rhizoctonia solani* on potato-dextrose agar and of soil temperature on the development of *Rhizoctonia* root cankers on alfalfa.

cankers on each tap root at a soil temperature of 30° C. Figure 2 shows the degree of root canker development at the different soil temperatures. From the figure it appears there are small cankers on the roots grown at a soil temperature of 40° C. but control plants grown at this same soil temperature, and not inoculated, had small cankers similar to those on the inoculated plants and they are considered to be a result of the soil temperature being too warm for healthy alfalfa plant growth. That a soil temperature of 40° C. was too warm for the plants was also evidenced by short and unhealthy-appearing top growth of the alfalfa.

As previously stated, root cankers, as observed in the field, develop during the warm part of the season. At Bard, California, in fields cropped

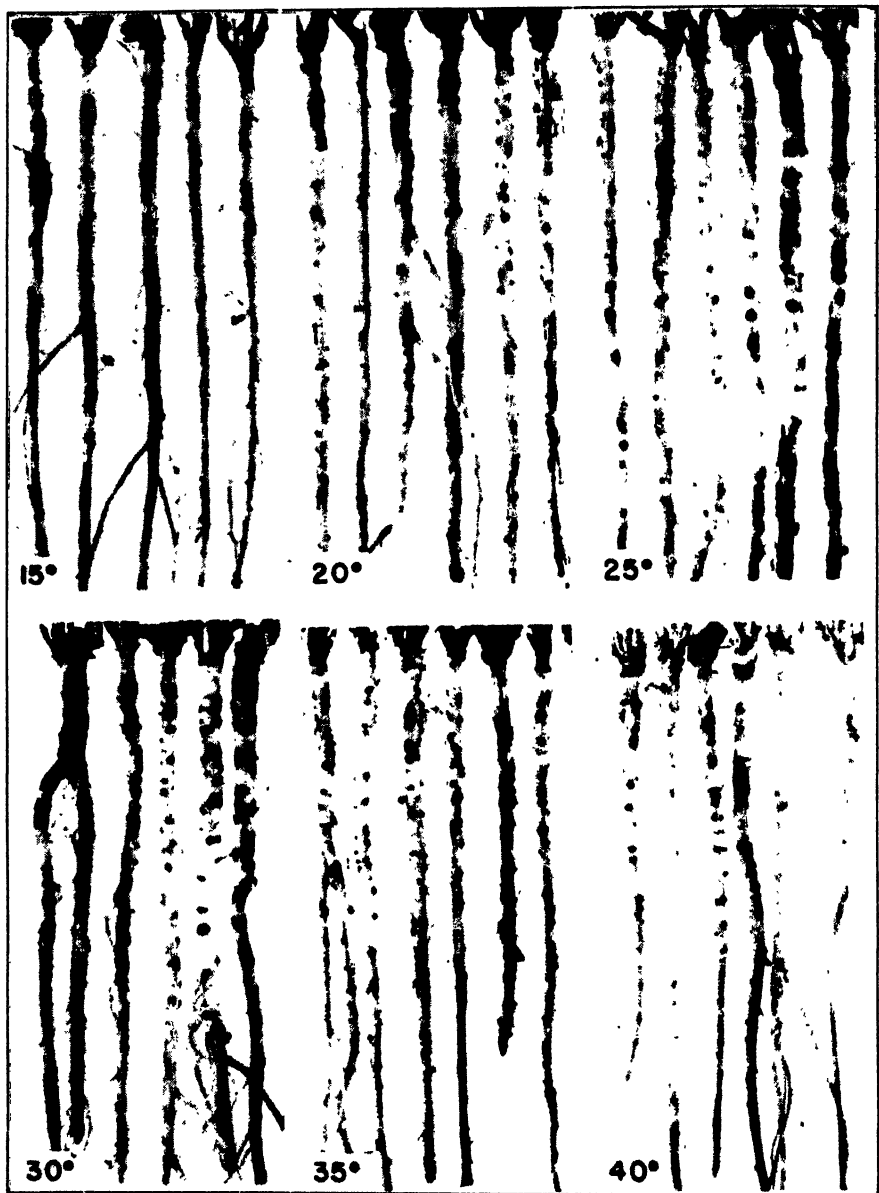


FIG. 2. Effect of soil temperature on the development of *Rhizoctonia* root canker of alfalfa. Roots were inoculated with *Rhizoctonia solani* and grown at the soil temperatures indicated for 50 days.

with alfalfa, the soil, at 3 inches below the surface, does not warm to 20° C. before about the middle of April and cools below 20° C. about the middle

of October (Fig. 3).⁴ During this period soil temperatures are above 20° C. and are favorable to disease development, as determined by controlled experiments (Fig. 1). Soil temperature is the main factor which influences the development of alfalfa *Rhizoctonia* root canker.

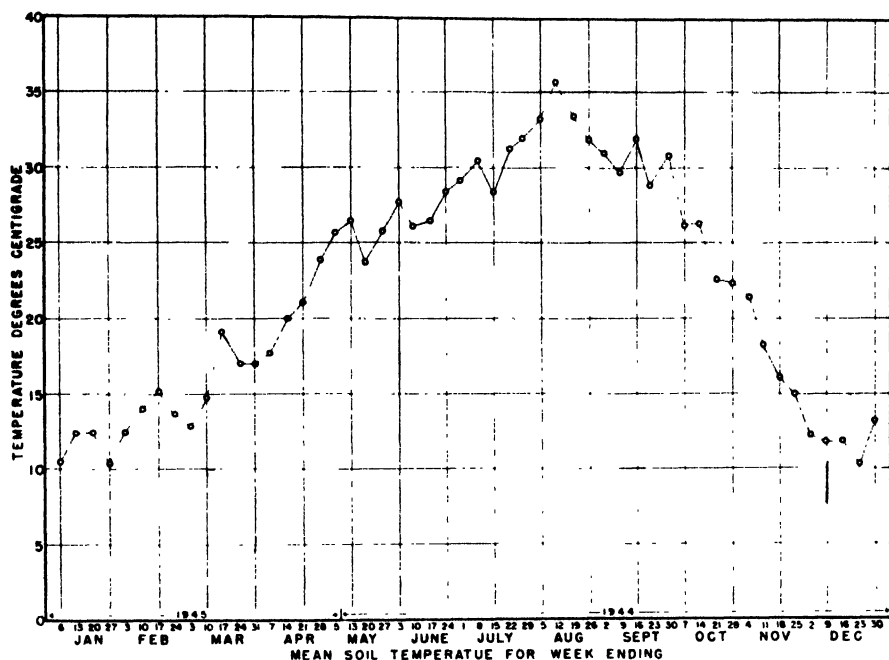


FIG. 3. Mean weekly soil temperature 3 inches below the surface at Bard, California, from May 7, 1944, to May 5, 1945.

SUMMARY

Optimum temperature for growth of *Rhizoctonia solani* on potato-dextrose agar was about 23–30° C. Optimum soil temperature for development of *Rhizoctonia* root canker of alfalfa was about 25–30° C. Very few cankers developed at a soil temperature below 20° or above 35° C. in 50 days.

In the field, very few root cankers develop before the soil warms to 20° C. or higher in the spring, and cankers cease to develop in the fall after the soil cools to approximately 20° C. or lower.

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⁴ Records of soil temperatures at Bard, California, were supplied by E. G. Noble, Agronomist, Division of Irrigation Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

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A COMPARISON OF THE EXPERIMENTAL HOST RANGES OF TOBACCO-ETCH AND TOBACCO-MOSAIC VIRUSES

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(Accepted for publication April 29, 1946)

Tests of the experimental host ranges of tobacco-etch virus (*Marmor erodens* H.) and tobacco-mosaic virus (*M. tabaci* H.) have been conducted over several years. The present paper summarizes briefly what has been learned from them and records the behavior of all species of plants that were inoculated during the experiments.

REVIEW OF LITERATURE

Tobacco-etch and tobacco-mosaic viruses have been reported as infecting only a few plant species in nature. *Nicotiana tabacum* L., *Lycopersicon esculentum* Mill., *Capsicum frutescens* L., *Physalis heterophylla* Nees, and *Petunia hybrida* Vilm. (12) have been found infected with each of the two viruses. In addition, tobacco-etch virus (under the name of "virus of mosaic C") has been recovered from naturally infected plants of *Datura stramonium* L., its first recognized host (4), and *Physalis subglabrata* Mackenzie and Bush (11); tobacco-mosaic virus has been recovered from naturally infected plants of *Solanum carolinense* L. (12). All of these species are of the family Solanaceae. A specialized strain of tobacco-mosaic virus has been reported as infecting *Plantago lanceolata* L. and *P. major* L. in an additional family, the Plantaginaceae (9).

Experimental studies have demonstrated a much wider potential range of hosts for both viruses. It has long been known that the typical strain of tobacco-mosaic virus is able to infect a large proportion of all inoculated species of solanaceous plants; indeed among many such species tested experimentally in the past only one, *Physalis viscosa* L., has been shown to be naturally immune (6). In this paper, evidence is presented that at least one other solanaceous species, *Cyphomandra betacea* Sendt. (See table 1), is immune from infection by rubbing inoculation, but it is evident that a great majority of solanaceous plants are susceptible to infection under appropriate circumstances. Fernow (4) was the first to show experimentally that tobacco-mosaic virus might cause disease in a representative of another family; he described its effects in *Martynia louisiana* Mill., of the Martyniaceae. Price (14) found some varieties of the common bean, *Phaseolus vulgaris* L., to be susceptible on inoculation and to be suitable substitutes for *Nicotiana glutinosa* L. of the Solanaceae in estimating concentrations of this virus. This added another, the Leguminosae, to the two families already known to include susceptible species. Grant (5) showed that potential hosts of this virus are in reality widely scattered among herbaceous dicotyledons, reporting 29 susceptible species in 14 families other than the Solanaceae. In a subsequent study by the writer, the ability of

plant species in many families to support the multiplication of tobacco-mosaic virus in their tissues, after its introduction by mechanical inoculation, was confirmed and this susceptibility was found to be correlated with taxonomic affiliations of the experimental hosts (7). One additional extension of the experimental host range of tobacco-mosaic virus has been provided by Severin (15), who found recently that guayule (*Parthenium argentatum* A. Gray of the family Compositae) developed small necrotic areas at the site of inoculation and supported a multiplication of virus locally.

Not only does the experimental host range of tobacco-mosaic virus far outrun the bounds of the Solanaceae, but that of tobacco-etch virus also transcends those limits. In the course of a previously reported investigation (10), aimed at securing a host that would show conspicuous primary lesions after inoculation with etch virus, many additional species were found susceptible. These species were not, however, specifically listed in the publication cited. In a brief abstract (11) it was reported that all species found capable of supporting the increase of tobacco-etch virus seemed also capable of supporting increase of tobacco-mosaic virus if inoculated but that the converse was not true. Complete data are given in the present paper.

MATERIALS AND METHODS

The strain of tobacco-mosaic virus used for the tests recorded in this paper was the green-mottling, distorting strain (var. *pulgaris* H.) that seems to be rather typical of tobacco-adapted isolates; the strain of tobacco-etch virus was that known as the severe-etch strain (var. *severum* H.), an isolate of remarkable constancy originally obtained from E. M. Johnson, who first described it (12). This severe-etch strain represents a naturally-occurring variant of the virus originally reported as the cause of a disease called "etched" by Vallean and Johnson (17) and later referred to as etch (12). Use of strains other than these two would have produced additional symptoms and otherwise modified the data here presented but could not be included without greatly altering the scope of the investigation.

Inoculum was prepared differently for the two viruses because of differences in infectivity and stability. A once-frozen and thawed sample of a 1:50 aqueous dilution of juice expressed from diseased tobacco plants was used as the tobacco-mosaic inoculum, to avoid retention of measurable amounts of virus on the surface of inoculated but not infected leaves. Freshly expressed whole juice from tobacco plants, usually obtained 10 days after infection, was used for severe-etch inoculum, except in a few cases in which similar undiluted juice expressed from infected plants of the species *Nicotiana glutinosa* was substituted for it.

All inoculations were made by rubbing the upper surfaces of leaves with pads of cheesecloth moistened with the above described inocula. Additional opportunity for entrance of virus was provided by 100 small pin punctures made through the moistened inoculated leaves to mark the areas of inoculation in each plant.

EXPERIMENTAL RESULTS

Evidence that the two viruses used in the present investigation are not closely related was obtained by observing their failure to protect against each other in suitable hosts. In figure 1, A is represented a leaf of *Physalis peruviana* Mill., mottled as a result of systemic infection by tobacco-mosaic virus yet bearing necrotic primary lesions caused by subsequent inoculation of the affected leaf with severe-etch virus; the necrotic lesions occurred both

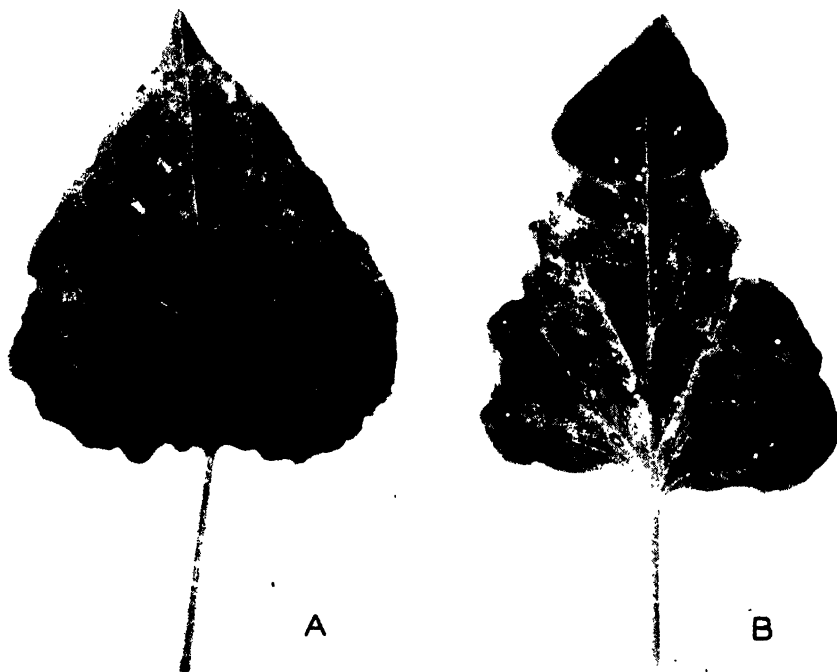


FIG. 1. A. Leaf of *Physalis peruviana*, mottled as a result of systemic infection by tobacco mosaic virus yet bearing numerous necrotic primary lesions as a result of subsequent inoculation with severe-etch virus. Most of the necrotic lesions occurred in chlorotic areas of the leaf, because green areas of the mottling pattern were relatively small. B. Leaf of *Nicotiana glutinosa*, similarly mottled as a result of systemic infection by severe-etch virus yet bearing necrotic primary lesions as a result of subsequent inoculation with tobacco-mosaic virus. (Photograph by J. A. Carlile.)

in such green areas as were present and in the more extensive chlorotic areas of the mottled leaf. In figure 1, B is represented a leaf of *Nicotiana glutinosa*, mottled and distorted as a result of systemic infection by severe-etch virus, yet bearing necrotic primary lesions formed as a result of subsequent inoculation with tobacco-mosaic virus; here also the necrotic lesions occurred both in the green blister-like areas and in the more extensive chlorotic areas of the mottled leaf. This failure of protection against formation

TABLE 1.—Results of inoculations made to compare experimental host ranges of tobacco-etch and tobacco-mosaic viruses

Family and species	Results of inoculation ^{a, b}	
	With tobacco-etch virus	With tobacco-mosaic virus
AIZOACEAE		
<i>Mesembryanthemum crystallinum</i> L.	0	1
<i>M. lineare</i> Thunb.	0	4 (L, V-O; S, V-CM)
<i>Mollugo verticillata</i> L.	1	1
<i>Tetragonia expansa</i> Murr.	3 (L, V-CSp; S, Va-O)	3 (L, V-CSp-Ny; S, Va-O)
AMARANTHACEAE		
<i>Amaranthus tricolor</i> L.	0	1
<i>Celosia argentea</i> L.	3 (L, V-CSp; S, Va-O)	4 (L, V-CSp; S, V-CSp-CM)
<i>Gomphrena haageana</i> Klotzsch	0	0
BALSAMINACEAE		
<i>Impatiens balsamina</i> L.	0	0
<i>I. holstii</i> Engler and Warb.	0	3 (L, V-CSp-NRiCo; S, Va-O)
CAMPANULACEAE		
<i>Campanula carpatica</i> Jacq.	0	0
<i>C. drabifolia</i> Sibth. and Sm.	2	2
<i>C. lactiflora</i> Bieb.	0	0
<i>C. medium</i> L.	0	0
<i>C. pyramidalis</i> L.	0	0
<i>C. rotundifolia</i> L.	0	0

^a Numbers have the following significance:

- 0, no increase of virus and no manifestation of disease (tested species insusceptible).
- 1, increase of virus locally but neither systemic spread of virus nor production of an obvious disease (tested species susceptible to localized masked disease).
- 2, virus detected in leaves other than those originally inoculated but no production of obvious disease (tested species susceptible to systemic masked disease).
- 3, local disease manifestations but no spread of virus (tested species susceptible to localized but obvious disease).
- 4, systemic spread of virus and obvious manifestations of disease (tested species susceptible to systemic and obvious disease).

^b Abbreviations used within explanatory parentheses in the table have the following significance:

Ab, abscission
 Bl, black
 Br, brown
 C, chlorosis or chlorotic
 Ch, chlorophyll-retention patterns
 Co, concentric patterns
 Col, collapse
 D, death of plant
 Dif, diffuse
 Dis, distortion or distorted
 E, etched patterns
 En, enations
 Ex, extreme
 Fed, flower color deepened
 G, green or greenish
 K, klendusic (often escaping infection)
 L, locally
 Lc, leaf or leaves
 M, mottling
 Ma, masking
 Mv, midvein

N, necrosis or necrotic
 Ny, necrosis with surrounding yellowing
 O, no manifestation of disease observed
 Pc, pale center
 Pe, petiole or petioles
 Pu, puckered
 R, red
 Ri, ring like spots
 S, systemically
 Sc, segments of rings
 Sp, spots
 St, stem or stems
 Str, streak
 Stu, stunting
 V, virus recoverable
 Va, virus absent
 Vc, veinclearing
 W, white
 Wi, wilting
 Y, yellowing or yellow
 Yo, young

TABLE 1.—(Continued)

Family and species	Results of inoculation ^{a, b}	
	With tobacco-etch virus	With tobacco-mosaic virus
CAPPARIDACEAE		
<i>Cleome spinosa</i> L.	0	1
<i>Polanisia trachysperma</i> Torr. and Gray	0	2
CARICACEAE		
<i>Carica papaya</i> L.	0	3 (L, V-CSp; S, Va-O)
CARYOPHYLLACEAE		
<i>Dianthus barbatus</i> L.	0	0
<i>D. chinensis</i> L.	0	0
<i>Gypsophila elegans</i> Bieb.	1	1
<i>Lychnis coeli-rosa</i> (L.) Desr.	0	1
<i>Silene anglica</i> L.	4 (L, V-NRi; S, V-NRi-E)	1
<i>S. pendula</i> L.	0	1
<i>Stellaria media</i> (L.) Cyrill.	0	1
CHENOPODIACEAE		
<i>Beta vulgaris</i> L.	0	3 (L, V-CSpRRi; S, Va-O)
<i>Chenopodium album</i> L.	0	3 (L, V-CSp-RNSp; S, Va-O)
<i>C. ambrosioides</i> L.	0	3 (L, V-CSp-NSp; S, Va-O)
<i>C. glaucum</i> L.	0	3 (L, V-CSp; S, Va-O)
<i>C. rubrum</i> L.	0	3 (L, V-CSp-LeAb; S, Va-O)
<i>Kochia scoparia</i> Schrad.	0	1
<i>Spinacia oleracea</i> L.	0	1
COMPOSITAE		
<i>Ageratum conyzoides</i> L.	0	0
<i>Ambrosia trifida</i> L.	0	1
<i>Arctium lappa</i> L.	0	1
<i>Bellis perennis</i> L.	0	3 (L, V-CSp; S, Va-O)
<i>Bidens frondosa</i> L.	0	1
<i>Brachycome iberidifolia</i> Benth.	4 (L, Va-O; S, V-CM)	2
<i>Calendula officinalis</i> L.	0	3 (L, V-CSp; S, Va-O)
<i>Callistephus chinensis</i> Nees	3 (L, V-BrNRi; S, Va-O)	1
<i>Centaurea americana</i> Nutt.	0	1
<i>C. montana</i> L.	0	1
<i>C. moschata</i> L.	0	0
<i>Charicis heterophylla</i> Cass.	4 (L, V-O; S, V-CSp)	1
<i>Chrysanthemum leucanthemum</i> L.	0	0
<i>Cichorium endivia</i> L.	0	3 (L, V-CCoRi; S, Va-O)
<i>Corcopsis grandiflora</i> Nutt.	0	3 (L, V-CSp; S, Va-O)
<i>Dahlia pinnata</i> Cav.	0	0
<i>Dimorphotheca aurantiaca</i> DC.	4 (L, V-BrNSp; S, V-BrNSp-CM-NStStr)	2
<i>Emilia flammica</i> Cass.	0	4 (L, V-O; S, V-CM-CSp)
<i>Erigeron annuus</i> (L.) Pers.	0	1
<i>E. speciosus</i> DC.	0	0
<i>Eupatorium lascaurii</i> Carr.	1	1
<i>Gaillardia aristata</i> Pursh	0	0
<i>Galinsoga parviflora</i> Cav.	0	1
<i>Gamolepis tagetes</i> DC.	2	1
<i>Helianthus annuus</i> L.	3 (L, V-NRi; S, Va-O)	1
<i>H. tuberosus</i> L.	0	0
<i>Helichrysum bracteatum</i> Andr.	0	3 (V-CSp; S, Va-O)

TABLE 1.—(Continued)

Family and species	Results of inoculation ^{a, b}	
	With tobacco-etch virus	With tobacco-mosaic virus
<i>Helioopsis scabra</i> Dun.	0	0
<i>Helipterum humboldtianum</i> DC.	1	1
<i>Leptosyne maritima</i> (Hook. f.) A. Gray	0	1
<i>Rudbeckia hirta</i> L.	0	3 (L, V-CSp; S, Va-O)
<i>Sanvilatia procumbens</i> Lam.	0	1
<i>Solidago rugosa</i> Mill.	0	0
<i>Tagetes erecta</i> L.	0	2
<i>T. patula</i> L.	0	1
<i>T. signata</i> Bartl.	0	1
<i>Taraxacum officinale</i> Weber	0	0
<i>Thelesperma hybridum</i> Voss	0	0
<i>Zinnia elegans</i> Jacq.	4 (L, V-O; S, V-CM)	1
<i>Z. haageana</i> Regel	0	2
CONVOLVULACEAE		
<i>Calonyction aculeatum</i> House	0	1
<i>Ipomoea batatas</i> Lam.	0	0
<i>I. nil</i> Roth	0	1
<i>I. setosa</i> Ker	0	0
<i>I. tricolor</i> Cav.	0	1
CRUCIFERAE		
<i>Barbarea vulgaris</i> R. Br.	0	0
<i>Brassica napobrassica</i> Mill.	0	1
<i>B. oleracea</i> L.	0	0
<i>B. pekinensis</i> Rupr.	0	0
<i>Capsella bursa-pastoris</i> (L.) Medic.	0	1
<i>Cheiranthus allionii</i> Hort.	0	1
<i>C. cheiri</i> L.	0	1
<i>Iberis gibraltaria</i> L.	0	1
<i>I. sempervirens</i> L.	0	0
<i>I. umbellata</i> L.	0	1
<i>Lobularia maritima</i> Desv.	0	1
<i>Lunaria annua</i> L.	0	4 (L, V-O; S, V-LeDis) or 2
<i>Mathiola bicornis</i> DC.	0	1
<i>M. incana</i> R. Br.	0	0
<i>Raphanus raphanistrum</i> L.	0	0
<i>R. sativus</i> L.	0	0
GERANIACEAE		
<i>Geranium carolinianum</i> L.	0	1
<i>Pelargonium hortorum</i> Bailey	0	0
<i>P. odoratissimum</i> Ait.	0	0
GESNERIACEAE		
<i>Saintpaulia ionantha</i> Wendl.	0	0
<i>Sinningia speciosa</i> Benth. and Hook.	0	1
HYDROPHYLLACEAE		
<i>Emmenanthe penduliflora</i> Benth.	4 (L, V-BrNSp; S, V-BrNSp-D)	2
<i>Nemophila insignis</i> Benth.	1	1
<i>N. maculata</i> Benth.	1	3 (L, V-BrNSpRi; S, Va-O)

TABLE 1.—(Continued)

Family and species	Results of inoculation ^{a, b}	
	With tobacco-etch virus	With tobacco-mosaic virus
<i>Phacelia campanularia</i>		
Gray	4 (L, V-O; S, V-Vc-CM)	4 (L, V-O; S, V-CSp)
<i>P. ciliata</i> Benth.	2	2
<i>P. grandiflora</i> (Benth.)		
Gray	4 (L, V-CSp; S, V-CM-Ma)	4 (L, V-O; S, V-CVc-CM)
<i>P. viscida</i> (Benth.) Torr.	4 (L, V-O; S, V-Vc-N- NSStStr)	4 (L, V-O; S, V-CM)
<i>P. whittlaria</i> Gray	4 (L, Va-O; S, V-NSe)	4 (L, V-O; S, V-CM)
LABIATAE		
<i>Colus blumei</i> Benth.	0	1
<i>Lamium amplexicaule</i> L.	4 (L, V-O; S, V-CM-Ma)	2
<i>Lycopus rubellus</i> Moench	0	1
<i>Marrubium vulgare</i> L.	0	1
<i> Melissa officinalis</i> L.	0	1
<i>Mentha spicata</i> L.	0	1
<i>Nepeta cataria</i> L.	0	1
<i>N. hederacea</i> (L.) Trev.	0	0
<i>N. mussini</i> Spreng.	0	1
<i>Prunella vulgaris</i> L.	0	1
<i>Salvia azurea</i> Lam.	0	1
<i>S. farinacea</i> Benth.	0	1
<i>S. patens</i> Cav.	0	1
<i>S. splendens</i> Ker	0	1
LEGUMINOSAE		
<i>Dolichos lablab</i> L.	0	0
<i>Glycine max</i> (L.) Merr.	0	0
<i>Lens culentata</i> Moench	0	0
<i>Lupinus hartwegii</i> Lindl.	0	0
<i>Medicago sativa</i> L.	0	0
<i>Phaseolus coccineus</i> L.	0	0
<i>P. aureus</i> Roxb.	0	0
<i>P. limensis</i> Macf.	0	0
<i>P. vulgaris</i> L. (according to variety)	0	3 (L, V-RNSp; S, Va-O) or 0
<i>Pisum sativum</i> L.	0	0
<i>Trifolium incarnatum</i> L.	0	0
<i>T. pratense</i> L.	0	0
<i>T. repens</i> L.	0	0
<i>Vicia faba</i> L.	0	0
<i>V. sativa</i> L.	0	0
<i>Vigna sinensis</i> (L.) Endl.	0	0
LOBELIACEAE		
<i>Lobelia erinus</i> L.	0	1
<i>L. gracilis</i> Andr.	4 (L, V-O; S, V-DifCM)	1
<i>L. inflata</i> L.	4 (L, V-O; S, V-CM)	1
<i>L. tenuior</i> R. Br.	4 (L, V-O; S, V-C-Wi-N)	1
MARTYNIACEAE		
<i>Proboscidea louisiana</i> (Mill.) Woot. and Stand.	4 (L, V-O; S, V-Vc-CM)	4 (L, V-O; S, V-CM)
NOLANACEAE		
<i>Nolana lanceolata</i> Miers	4 (L, V-O; S, V-CVc-N- Stu-CM-Dis-LePu)	4 (L, V-O; S, V-CM)
PAPAVERACEAE		
<i>Chelidonium majus</i> L.	0	3 (L, V-DifC; S, Va-O)
<i>Hannemannia fumariaefolia</i> Sweet	0	0
<i>Papaver nudicaule</i> L.	0	3 (L, V-C-N; S, Va-O)
<i>P. orientale</i> L.	0	0

TABLE 1.—(Continued)

Family and species	Results of inoculation ^{a, b}	
	With tobacco-etch virus	With tobacco-mosaic virus
PHYTOLACCACEAE		
<i>Phytolacca dceandra</i> L.	0	3 (L, V-CRi-RRi; S, Va-O)
PLANTAGINACEAE		
<i>Plantago lanceolata</i> L.	0	1
<i>P. major</i> L.	0	3 (L, V-NBrRi; S, Va-O)
<i>P. rugelii</i> Dene.	0	3 (L, V-CSp; S, Va-O)
POLEMONIACEAE		
<i>Cobaea scandens</i> Cav.	0	0
<i>Gilia capitata</i> Dougl.	0	1
<i>G. liniflora</i> Benth.	0	2
<i>Phlox drummondii</i> Hook.	0	4 (L, V-CSp-CCoRi; S, V-CSp)
PORTULACACEAE		
<i>Portulaca oleracea</i> L.	3 (L, V-LeWiAb; S, Va-O)	1
PRIMULACEAE		
<i>Anagallis arvensis</i> L.	0	1
<i>Primula malacoides</i> Franch.	2	1
<i>P. obconica</i> Hance	0	1
SCROPHULARIACEAE		
<i>Antirrhinum majus</i> L.	0	1
<i>Collinsia bicolor</i> Benth.	3 (L, V-CSp; S, Va-O)	4 (L, V-O; S, V-CM)
<i>Cymbalaria muralis</i> Gaertn., Mey. and Scherb.	1	1
<i>Digitalis purpurea</i> L.	0	4 (L, V-NSe; S, V-O)
<i>Linaria maroccana</i> Hook. f.	2	1
<i>L. vulgaris</i> Hill	0	1
<i>Mimulus moschatus</i> Dougl.	0	1
<i>M. tigrinus</i> Hort.	0	1
<i>Nemesia strumosa</i> Benth.	0	2
<i>Penstemon grandiflorus</i> Nutt.	1	1
<i>Torenia fournieri</i> Lind.	1	2
<i>Verbascum phoeniceum</i> L.	0	1
<i>Veronica longifolia</i> L.	0	1
<i>V. officinalis</i> L.	0	1
<i>V. peregrina</i> L.	0	1
<i>Zaluzianskya villosa</i> Schmidt	4 (L, V-CSp; S, V-O)	2
SOLANACEAE		
<i>Browallia americana</i> L.	0	3 (L, V-CSp-CRi-Ch; S, Va-O)
<i>B. speciosa</i> Hook.	4 (L, V-O; S, V-CM-Stu)	4 (L, V-CSp; S, V-C-CM) or 3 (L, V-BrNSpPe-LeAb; S, Va-O)
<i>Capsicum frutescens</i> L.	4 (L, V-O; S, V-CM)	4 (L, V-CSp-Ch; S, V-C-Stu-CM-Ch) or 4 (L, V-CSp-NSp-LeAb; S, V-CSp-NSp) or 3 (L, V-NSp-LeAb; S, Va-O) or 3 (L, V-CSp-NSp-LeAb; S, Va-O)
<i>Cyphomandra tetacea</i> Sendt.	0	0
<i>Datura stramonium</i> L.	4 (L, V-O; S, V-CM-Dis)	3 (L, BrNSpPe; S, Va-O)
<i>Lyium chinense</i> Mill.	3 (L, V-DifCSp or BrNSp; S, Va-O)	3 (L, V-DifCSp; S, Va-O)
<i>Lycopersicon chilense</i> Dun.	0	4 (L, V-O; S, V-CM-K)

TABLE 1.—(Continued)

Family and species	Results of inoculation ^{a, b}	
	With tobacco-etch virus	With tobacco-mosaic virus
<i>L. esculentum</i> Mill.	4 (L, V-O; S, V-CM)	4 (L, V-O; S, V-CM-Dis)
<i>L. hirsutum</i> Dun.	2	2
<i>L. peruvianum</i> (L.) Mill.	4 (L, V-O; S, V-CSp)	1
<i>L. pimpinellifolium</i> (Jusl.) Mill.	4 (L, V-O; S, V-CM)	4 (L, V-O; S, V-CM) or 2
<i>Nicandra physalodes</i> (L.) Pers.	4 (L, V-O; S, V-CM-CSp)	4 (L, V-O or CSp or Ch; S, V-CSp or Ch or CM- Fed)
<i>Nicotiana acuminata</i> (Grah.) Hook.	4 (L, V-C; S, V-CVe-C- Stu-CM)	4 (L, V-BrNSp; S, V-N- StStr) or 3 (L, V-BrNSp; S, Va-O)
<i>N. alata</i> Link and Otto	4 (L, V-O; S, V-Ve-CM-E)	4 (L, V-BI BrNSpPc; S, V-N or L, V-CSp; S, V-CM)
<i>N. bigelovii</i> S. Wats.	4 (L, V-O; S, V-C-Pu-Stu- LeDis-CM)	4 (L, V-CSp or O; S, V-C- Stu-CM or D)
<i>N. bonariensis</i> Lehm.	4 (L, V-DifCSp; S, V-CVe- DifCSp)	4 (L, V-CSp; S, V-CVe- CM-Dis)
<i>N. candigera</i> Phil.	4 (L, V-Y-N; S, V-CM- Stu)	4 (L, V-CNSp; S, V-C-Pu- Stu-CM-Dis)
<i>N. clelandii</i> A. Gray	4 (L, V-O; S, V-Stu-CVe- CM-E)	4 (L, V-C; S, V-YoLePu- C-CM)
<i>N. digluta</i> Clausen and Goodspeed	4 (L, V-O; S, V-Ve-CM- Dis)	3 (L, V-BrNSpPc; S, Va-O)
<i>N. glauca</i> Grah.	1	4 (L, V-CSp or O; S, V-CM) or 3 (L, V-CSp; S, Va-O) or 2 or 1
<i>N. glutinosa</i> L.	4 (L, V-CSp; S, V-CM-E)	4 (L, V-BrNSpPc; S, V-NSp) or 3 (L, V-BrNSpPc; S, Va-O)
<i>N. langsdorffii</i> Weinm.	4 (L, V-O; S, V-Ve-GCM)	4 (L, V-BINSpPc; S, V-N- StStr)
<i>N. longiflora</i> Cav.	4 (L, V-O; S, V-Ve-GCM)	4 (L, V-O; S, V-Stu-CM- Dis)
<i>N. nudicaulis</i> S. Wats.	4 (L, V-O; S, V-CM-Dis)	4 (L, V-CSp; S, V-C-Stu)
<i>N. otophthora</i> Griseb.	0	4 (L, V-O; S, V-CM-Stu)
<i>N. palmeri</i> A. Gray	0	4 (L, V-C; S, V-CVe- LeYPu-CM)
<i>N. paniculata</i> L.	4 (L, V-CSp; S, V-CRi)	4 (L, V-CSp; S, V-CVe- CM-En)
<i>N. plumbaginifolia</i> Viv.	4 (L, V-O; S, V-CVe-CM)	4 (L, V-O; S, V-CVe-C- CM-Dis)
<i>N. raimondii</i> Macbride	0	4 (L, V-O; S, V-CSp-CM) or 2
<i>N. repanda</i> Willd.	4 (L, V-CSp or NSe; S, V- CM-CSp)	4 (L, V-BrNSpPc; S, V- N-D)
<i>N. rustica</i> L.	4 (L, V-O; S, V-Ve-CM- Dis)	4 (L, V-BrNSpPc-Ny; S, V-NSp)
<i>N. sandrac</i> Hort.	4 (L, V-DifCSp; S, V- CVeE-CM)	4 (L, V-BINSpPc; S, V-N or L, V-CSp; S, V-CM)
<i>N. solanifolia</i> Walp.	4 (L, V-O; S, V-CSp)	4 (L, V-O; S, V-CM)
<i>N. sylvestris</i> Spegaz. and Comes	4 (L, V-O; S, V-CM)	4 (L, V-O; S, V-CM)
<i>N. tabacum</i> L.	4 (L, V-CSpE; S, V-CM-E)	4 (L, V-CSp; S, V-CVePu- CM-Dis)

TABLE 1.—(Concluded)

Family and species	Results of inoculation ^{a, b}	
	With tobacco-etch virus	With tobacco-mosaic virus
<i>N. tomentosa</i> Ruiz and Pav.	4 (L, V-O; S, V-CSp)	4 (L, V-CSp; S, V-CSp-CM-Dis-En)
<i>N. tomentosiformis</i> Good-speed	0	4 (L, V-CSp; S, V-CM-CSp-En)
<i>N. trigonophylla</i> Dun.	4 (L, V-O; S, V-CM)	4 (L, V-CSp-Ch; S, V-CVe-C-CM-Dis-Stu)
<i>N. undulata</i> Ruiz and Pavon	4 (L, V-C-LeCol; S, V-Stu-C-CM-LeCol-D)	4 (L, V-NSp; S, V-N or L, V-C; S, V-CVe-CM-Dis-Stu)
<i>N. wigandioides</i> Koch and Fint	0	1
<i>Nierembergia hippomanica</i> Miers	1	4 (L, V-O; S, V-CM-Dis)
<i>Petunia hybrida</i> Vilm.	4 (L, V-O; S, V-CM-Dis)	4 (L, V-O; S, V-C-CM)
<i>Physalis alkekengi</i> L.	1	4 (L, V-O; S, V-CM) or 2
<i>P. angulata</i> L.	4 (L, V-NSp; S, V-CM-Dis)	4 (L, V-WNRi-LeAb; S, V-N-LeAb)
<i>P. peruviana</i> L.	4 (L, V-BrNSpPe; S, V-NSp-Wi-LeAb-CM or D)	4 (L, V-O; S, V-CM)
<i>P. subglabrata</i> Mackenzie and Bush	2	2
<i>Salpiglossis sinuata</i> Ruiz and Pavon	4 (L, V-CSp; S, V-ExStu-LePu-CM)	4 (L, V-C-N; S, V-C-NSStStr-D)
<i>Schizanthus pinnatus</i> Ruiz and Pavon	4 (L, V-O; S, V-C-N-YoLeCol)	4 (L, V-NSp-PeStStStr; S, V-Stu-Wi-N-NSSt-D)
<i>Solanum capsicastrum</i> Link	1	3 (L, V-BrNSp; S, Va-O)
<i>S. dulcamara</i> L.	0	1
<i>S. integrifolium</i> Poir	1	4 (L, V-BrNSp-StN; S, V-BrNSp-PeNStr)
<i>S. melongena</i> L.	1	4 (L, V-CSp; S, V-CM) or 4 (L, V-BrNSp; S, V-N-StPeMvNSt-D) or 3 (L, V-BrNSp; S, Va-O)
<i>S. nigrum</i> L.	4 (L, V-O; S, V-CM)	4 (L, V-O; S, V-GCM)
<i>S. sanitwongsei</i> Craib	0	4 (L, V-O; S, V-Ch) or 2
<i>S. tuberosum</i> L.	4 (L, V-O; S, V-CM)	4 (L, V-N; S, V-N)
UMBELLIFERAE		
<i>Apium graveolens</i> L.	0	1
<i>Daucus carota</i> L.	0	1
<i>Pastinaca sativa</i> L.	0	0
<i>Petroselinum hortense</i> Hoffm.	0	1
<i>Trachymene caerulea</i> R. Grah.	0	0
VERBENACEAE		
<i>Lantana camara</i> L.	0	0
<i>Verbena canadensis</i> Britt.	0	1
<i>V. hybrida</i> Voss.	2	3 (L, V-CSp or NSe; S, Va-O)
<i>T. r. nosa</i> Gill. and Hook.	0	0

of necrotic primary lesions by one virus in areas already systemically involved in patterns of chlorotic mottling by the other would be expected from our knowledge of other dissimilarities between the two viruses but has not been specifically demonstrated heretofore.

In table 1 and in the text immediately following this paragraph, species names of plants for which complete data with respect to susceptibility to infection by both tobacco-etch and tobacco-mosaic viruses became available during the course of the study have been arranged alphabetically by families. Insusceptibility to either virus is indicated in the appropriate column of the table by the symbol 0, susceptibility by numbers 1 to 4, indicating different grades of disease as explained in a footnote to the table; details of recovery of virus and of manifestations of disease at the site of inoculation and in the growing tops of inoculated plants are described parenthetically where needed, by a set of symbols patterned on those of Grant (5) and Wellman (18). These symbols in parentheses, explained in a second footnote of the table, are used primarily for brevity but permit also an adequacy of detail that should facilitate selection of appropriate hosts for specified reactions desired in experimental work. Reisolation after 10 days of any etch virus or of enough tobacco-mosaic virus to produce at least 10 lesions on an inoculated plant of *Nicotiana glutinosa* forms the basis for indication of virus recovery in the table. Experience has shown that such criteria are, in general, appropriate for distinguishing potentially susceptible from insusceptible species. It is to be understood, of course, that a species appearing insusceptible might be infected eventually if attempts to inoculate it mechanically were continued or if grafting or other additional means of inoculation were used, but it is not known that this actually would occur. Obviously, too, a species found potentially susceptible, as shown by experimental infection after mechanical inoculation, may or may not ordinarily become infected in nature.

Families in which all tested species were found insusceptible to infection by both tobacco-etch and tobacco-mosaic viruses were the following (the tested species are also indicated): ACANTHACEAE—*Thunbergia alata* Bojer; AMARYLLIDACEAE—*Hippeastrum puniceum* Urban; APOCYNACEAE—*Vinca rosea* L.; ARACEAE—*Symplocarpus foetidus* (L.) Nutt.; ASCLEPIADACEAE—*Asclepias curassavica* L., *A. syriaca* L.; BEGONIACEAE—*Begonia semperflorens* Link and Otto; CUCURBITACEAE—*Citrullus vulgaris* Schrad., *Cucumis sativus* L., *C. melo* L., *Cucurbita maxima* Duchesne, *C. pepo* L., *Luffa acutangula* Roxb., *Momordica charantia* L.; DIPSACEAE—*Scabiosa atropurpurea* L., *S. caucasica* Bieb.; EUPHORBIACEAE—*Acalypha virginica* L., *Euphorbia heterophylla* L., *E. marginata* Pursh, *E. preslii* Guss., *Ricinus communis* L.; GRAMINEAE—*Avena sativa* L., *Holcus sudanensis* (Stapf) Bailey, *Hordeum vulgare* L., *Secale cereale* L., *Triticum aestivum* L., *Zea mays* L.; HYPERICACEAE—*Hypericum boreale* (Britton) Bicknell; LILIACEAE—*Allium cepa* L., *Lilium philippinense* Baker; LINACEAE—*Linum flavum* L.,

L. grandiflorum Desf., *L. perenne* L.; MALVACEAE—*Althaea rosea* Cav., *Gossypium hirsutum* L., *Hibiscus esculentus* L., *H. manihot* L., *Lavatera trimestris* L., *Malope trifida* Cav., *Malva rotundifolia* L.; NYCTAGINACEAE—*Mirabilis jalapa* L.; ONAGRACEAE—*Clarkia elegans* Dougl., *Oenothera lamareckiana* Ser.; OXALIDACEAE—*Oxalis corniculata* L., *O. stricta* L.; RANUNCULACEAE—*Aquilegia caerulea* James, *Delphinium cultorum* Voss, *Ranunculus asiaticus* L.; RESEDACEAE—*Reseda odorata* L.; ROSACEAE—*Geum canadense* Jacq., *G. chilense* Balb., *Potentilla arguta* Pursh, *P. monspeliensis* L., *Rosa odorata* Sweet; VIOLACEAE—*Viola arvensis* Murr.

Families in which all tested species were found insusceptible to tobacco-etch virus and in which all tested species showed increase of tobacco-mosaic virus locally but neither systemic spread of virus nor production of an obvious disease were the following (the tested species are also indicated): BIGNONIACEAE—*Incarvillea variabilis* Batalin; BORAGINACEAE—*Anchusa azurea* Mill., *A. capensis* Thunb., *Cynoglossum amabile* Stapf and Drummond, *Echium vulgare* L., *Heliotropium corymbosum* Ruiz and Pav., *Myosotis scorpioides* L., *M. sylvatica* Hoffm.; CRASSULACEAE—*Kalanchoë daigremontiana* Hamet and Perrier; FUMARIACEAE—*Adlumia fungosa* Greene; PLUMBAGINACEAE—*Limonium bonduelli* (Lest.) Kuntze, *L. sinuatum* (L.) Mill.; POLYGONACEAE—*Fagopyrum esculentum* Gaertn., *Polygonum hydropiper* L., *Rumex crispus* L., *R. obtusifolius* L.; TROPAEOLACEAE—*Tropaeolum majus* L.

It will be observed, by references to the digits in the body of table 1, that in no instance did a plant show evidence of infection and virus multiplication (numbers 1 to 4) by reason of mechanical inoculation with tobacco-etch virus and failure (indicated by 0) to become infected when similarly inoculated with tobacco-mosaic virus. However, the grades of disease were not so clearly correlated, one virus sometimes outrunning the other in moving throughout the plant (as shown by combinations of grade-of-disease numbers such as 1-4, 4-1) or outdoing the other in injury to invaded tissues (1-3, 3-1). Whatever one's estimate of the practical importance of masked carriers as virus reservoirs, a further importance attaches to them from a theoretical viewpoint. Presumably masked carriers of a virus, equally with obviously diseased plants, reflect at least the minimal biological requirements for increase of the virus within its host. It is true that severity of disease has been considered by Leach (13) to influence insect vectors in their transmission of viruses from diseased to healthy plants. Thus the perpetuation of viruses in nature may be dependent in some measure on degree of severity of disease manifestations. Yet plants obviously affected by disease after infection together with those in which virus multiplies but in which effects on the plant are so slight as to be overlooked on casual examination must be collectively recognized as defining, for theoretical purposes, the distribution in nature of materials and conditions essential for viral increase.

Reference to figure 2 will show that the experimental host range of etch

virus is centered about a part of the plant kingdom near the Solanaceae, much as is that of the mosaic virus; further, that it is narrower than, and enclosed within, that of the mosaic virus as has been noted in discussing table 1. Although all known naturally infected hosts of etch virus are members of the Solanaceae, it must be noted that the Hydrophyllaceae as a group are notable for susceptibility so far as tested experimentally. Widespread cultivation of solanaceous plants perhaps has been the decisive factor in drawing our attention to etch disease, but the causative virus long may have utilized wild species in the Solanaceae and in other families as hosts, and infections in nature may yet be found in hydrophyllaceous species or in closely related groups.

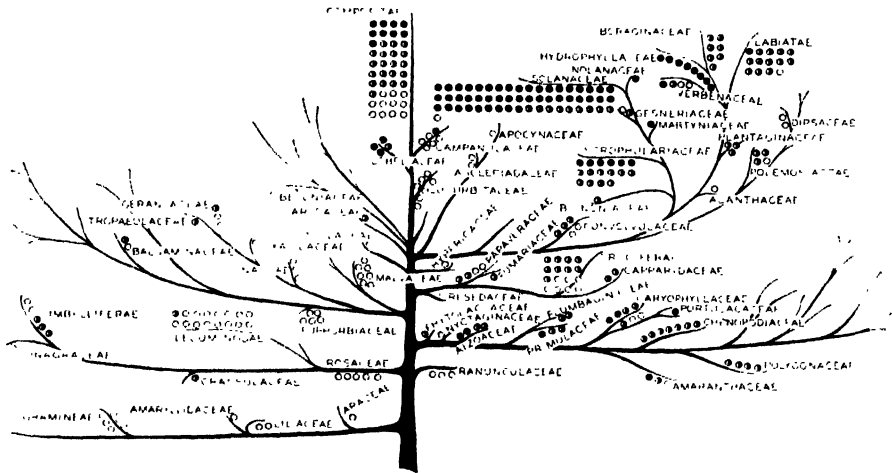


FIG. 2. Taxonomic relationships of plants susceptible to infection by tobacco-etch and tobacco-mosaic viruses. Species tested by inoculation are indicated by circles; black circles indicate species found susceptible to both viruses; white circles, to neither virus; half black circles, to tobacco mosaic, but not to tobacco-etch virus; no species was found susceptible to tobacco etch virus yet not to tobacco-mosaic virus.

Figure 2 emphasizes the fact that the experimental host ranges of tobacco-etch virus and of tobacco-mosaic virus are on the whole familial in character and tend to involve large blocks of species in closely related families, exceptional instances of resistance within these blocks being few, exceptional instances of susceptibility in other families being likewise few. Arrangement of families in the diagram is like that in diagrams used in an earlier paper (7) dealing with experimental hosts of tobacco-mosaic virus alone.

In table 2 there is a brief summary of the incidence of various types of response among the tested species. From this tabulation may be derived three generalizations other than the principal one that species incapable of supporting the increase of tobacco-mosaic virus proved incapable also of supporting the increase of tobacco-etch virus. These are: Species that showed systemic symptoms (Class 4) with mosaic virus mostly showed systemic symptoms with etch virus also; the reason for this is not evident, but the converse was true as well, for species that showed systemic symptoms

with etch virus mostly showed systemic symptoms with the mosaic virus as well. Species that did not take etch but did take mosaic tended to take mosaic mildly; why should this have been? Finally, species that took mosaic mildly mostly did not take etch.

Inspection of the classes of response (0 to 4) listed in table 1 and accompanying text suggests that for both viruses natural immunity must be considered the commonest of the five arbitrarily defined conditions of herbaceous plants in general. For tobacco-mosaic virus, the other four types of response in order of frequency were 1, 4, 3, 2, the largest number of susceptible plants localizing virus without obvious disease manifestations. For tobacco-etch virus, however, the order of frequency was 4, 1, 2, 3, most susceptible plants permitting systemic spread of virus and clearly showing disease. If the production of obvious systemic disease had been regarded

TABLE 2.—*Number of species affected by different grades of disease after testing separately with tobacco-etch and tobacco-mosaic inoculations*

Grades of disease ^a	0 (etch)	1 (etch)	2 (etch)	3 (etch)	4 (etch)
0 (mosaic)	111	0	0	0	0
1 (mosaic)	80	7	3	3	7
2 (mosaic)	5	1	4	0	5
3 (mosaic)	20	2	1	2	2
4 (mosaic)	11	5	0	2	39 ^b

^a Symbols 0 through 4 for arbitrary grades of disease as in table 1.

^b In a few instances plants of a given species have reacted at times with one grade of disease, but at other times more severely; the more severe grade is entered in this table for each such case.

as the only criterion of infection, it would have been concluded that the two viruses affected about equal numbers of the species inoculated (53 for severe-etch virus; 57 for tobacco-mosaic virus). Actually, however, this is far from the case when the more tolerant and the movement-restraining hosts are also recognized, 73 per cent of tested species proving naturally immune on inoculation with etch virus, only 36 per cent on inoculation with tobacco-mosaic virus.

Altogether, among the 310 species for which comparative data have been obtained, 83 proved susceptible to infection by both viruses, 116 to infection by tobacco-mosaic and not tobacco-etch virus, none to infection by tobacco-etch and not tobacco-mosaic virus, 111 to infection by neither virus.

DISCUSSION

On the basis of the present results, it seems feasible to predict that species susceptible to infection by strains of etch virus will usually, perhaps always, prove susceptible to infection by strains of tobacco-mosaic virus.

Such predictability characterizes no other pair of viruses that are serologically unrelated, confer no cross immunity, and have radically different resistances to inactivation by heat.

What is the meaning of this finding that all 83 species that proved susceptible to infection by etch virus were able to support increase of tobacco-mosaic virus? Are the requirements of these two viruses definitely related as has been suggested with respect to the requirements of some viruses (1, p. 117)? How should we explain the ability of tobacco-mosaic virus to infect an additional 116 species that appear to be naturally immune to infection with etch virus?

The experimental evidence now in hand suggests the hypothesis that the tested (severe-etch) strain of tobacco-etch virus requires one or more substances or conditions in its hosts in addition to all those needed by the strain used to represent tobacco-mosaic virus. In the course of time this hypothesis will be tested automatically, as information on additional species accumulates. It may be worthwhile to remark that exceptions naturally must be expected to arise in connection with any such generalization. If they prove few, however, they may tend merely to emphasize the significance of the rule without disproving it, for they may point only to independent relationships, such as specific harmful effects on tobacco-mosaic virus even in the presence of all that is normally required for its increase.

A greater similarity than might have been anticipated was found in the types of disease induced by tobacco-etch and tobacco-mosaic viruses in the experimental hosts as a group. The most distinctive characteristic of etch disease in tobacco, a whitish stippling of areas of leaf surface involved in primary and secondary lesions, has given rise both to the name of the causative virus (17, 12) and to a tendency to consider the virus as generically distinct from tobacco-mosaic virus (16). Reference to table 1, however, will show that chlorotic mottling (CM) was commonly observed in this study in systemically susceptible hosts, etching (E) very rarely. Etch virus appears, on the whole, to be a typical mosaic-type virus, rarely deviating even to the extent of producing etch patterns. Indeed the diseases induced by the two viruses seem fundamentally alike, and are often strikingly similar in their manifestations.

With this similarity of disease type and the partial predictability of host range in mind, one might tend to conclude that the two viruses were closely related genetically. Evidence against any very close relationship, however, may be summarized as follows: No interference with production of tobacco-mosaic virus was noted by Bawden and Kassanis in tests involving previous inoculation of plants with severe-etch virus (1, p. 113). No protection against local necrosis is afforded by inoculation of appropriate plants with one before the other (Fig. 1). There is no known antigenic similarity (3) despite an early erroneous report (2). Moreover, the two viruses differ sharply in their thermal stabilities: whereas tobacco-mosaic virus must be held at about 92° C. for 10 minutes to be completely inactivated, severe-etch

virus is inactivated at about 53° to 55° C. in the same length of time (1, p. 115; 10). Such numerous and fundamental differences would suggest a distant rather than a close relationship, if one exists.

In view of the evidence above detailed and because no other two viruses of wide host range are known to have so predictable a relationship between their hosts, it may be most feasible for the present to consider these two viruses with their respective strains and substrains as fairly closely allied, but distinct, members of the group of typical mosaic-disease viruses. This is already implied by their inclusion in a common genus as separate species (8).

SUMMARY

Among 310 plant species tested by inoculation with tobacco-etch and tobacco-mosaic viruses, 83 proved capable of supporting increase of both viruses, 111 proved incapable of acting as hosts of either virus, 116 were susceptible to attack by the mosaic though not by the etch virus, but none were susceptible to infection by the etch that were not susceptible to infection by the mosaic virus.

Types of disease induced in susceptible species are briefly recorded.

It is suggested that inclusion of the whole known potential host range of tobacco-etch virus within that of tobacco-mosaic virus may mean that the requirements of the former include all the requirements of the latter as well as some other requirement or requirements.

The earlier finding that plants susceptible to infection by tobacco-mosaic virus are largely confined to closely related families is confirmed. A similar but somewhat narrower host range is indicated for tobacco-etch virus.

Tobacco-etch and tobacco-mosaic viruses, though producing similar mottling diseases in many plants, including tobacco, are believed not to be closely related because they are immunologically and serologically distinct and definitely unlike in thermal stability. No other two viruses of so wide host range are known, however, to have so predictable a relation with respect to what plants they can infect. It is suggested that the similarity in their host requirements (which by no means amounts to an identity even in this characteristic), taken in conjunction with the general similarity of their induced diseases, may be considered as evidence of a relationship of a somewhat more distant type than that displayed by varieties of a single virus.

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HELMINTHOSPORIUM TURCICUM LEAF BLIGHT OF CORN

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The 1942 epidemic of *Helminthosporium turcicum* leaf blight came at a time when hybrid corn was largely replacing the old open-pollinated varieties. Many new lines and crosses were being developed and tested under different environmental conditions. Some were severely injured by leaf blight while other lines and crosses appeared to be more resistant. The double crosses in use in the Corn Belt were relatively susceptible. The differences observed indicated the advisability of further tests for resistance and of selecting resistant lines for use in the corn breeding program.

In 1944, as a result of field inoculations at Beltsville, Maryland, infection was produced that was even heavier than that in 1942. Some of the same lines and crosses were grown in both years. The results of the 1944 inoculations are given here in comparison with the results of natural infection in 1942.

METHODS OF INOCULATION

The 1944 field inoculations at Beltsville were made on 200 inbred lines, 176 single crosses, and 184 double crosses, assembled from the corn-growing areas, to test their relative susceptibility to leaf blight. These lines and crosses were grown in single-row plots of approximately 24 plants each in 3 or 4 replications, altogether covering about 5 acres.

Five out of 70 isolations of *Helminthosporium turcicum* Pass., from naturally infected inbred lines of dent corn grown in 1942 at Beltsville, were selected for rapid growth of mycelium, abundant spore production, and virulence. Single-spore cultures of all 5 were combined for the inoculations.

The cultures first were grown on oat hulls plus a small amount of corn meal in Petri dishes; and oat hulls transferred to potato-dextrose agar plates for spore production. An abundance of spores developed in about 3 weeks. Spore suspensions were made by running ten, 100-mm. Petri-dish cultures in one liter of water through an homogenizer¹ for 2 minutes. This suspension was stored at 5° C. until needed. The suspension was diluted 1 to 10 in the field and about 10 ml. sprayed into the central coil of leaves of each plant. Inoculations were begun June 23 when the plants were 12 to 18 inches tall and repeated about twice weekly until the plants began to tassel about the middle of July.

RESULTS OF THE INOCULATIONS

The final development of leaf blight, following inoculations in 1944, was even heavier than the epidemic of natural infection in 1942. Within a week after each inoculation, numbers of small water-soaked, yellow dots, 1 to 2 mm. in diameter, appeared in bands 2 to 3 inches wide, on the de-

¹ Manufactured by Eppenback, Inc., Long Island City, New York, Serial No. 3923.

veloping leaves. On some lines of corn these developed into typical *Helminthosporium turcicum* lesions. On other lines no further development took place. Until the last week in July little, if any, spreading of the disease from these original infections could be observed. For nearly a month following the first inoculation on June 23, there was almost no rain. Rains of 0.46 inches and 1.08 inches fell July 13 and July 20, respectively. On August 3, more than 5 inches of rain fell. Heavy dews formed at night. Leaf blight began to spread rapidly and continued to spread over all corn on the Station farm in the vicinity of the inoculation plots during the remainder of the growing season.

It had been hoped that the leaf blight would develop sufficiently from the initial inoculations so that plants, in segregating progenies, could be



FIG. 1. Scale for rating *Helminthosporium turcicum* leaf blight. 0.5, Very slight infection, one or two restricted lesions on lower leaves; 1, Slight infection, a few scattered lesions on lower leaves; 2, Light infection, moderate number of lesions on lower leaves; 3, Moderate infection, abundant lesions on lower leaves and few on middle leaves; 4, Heavy infection, lesions abundant on lower and middle leaves and extending to upper leaves; 5, Very heavy infection, lesions abundant on all leaves, plants may be prematurely killed.

classified as to susceptibility by the time they flowered, and thus lighten the task of hand pollination in the program of breeding for resistance. Differences among strains of corn and among plants in the amounts of infection that developed directly from inoculation, however, were entirely unsatisfactory for this purpose. Fortunately the disease started to spread during tasseling and the infection from this secondary spread was sufficiently heavy at pollinating time to permit a reasonably accurate selection of resistant plants for breeding operations.

The amount of blight infection was recorded in 6 classes (Fig. 1).² Plants with only a trace of infection were rated 0.5 and those with pro-

² Ullstrup, A. J., P. E. Hoppe, and Charlotte Elliott. Report of the committee on methods for reporting corn disease ratings. U. S. Dept. Agr., Agr. Res. Admin., Bur. Plant Indus., Soils, and Agr. Engin., Div. Cereal Crops and Dis. 23°C, 5 pp., Feb., 1945. [Processed.]

gressively heavier infection were rated 1, 2, 3, 4, or 5, the 5 representing very heavy infection. Ratings were made on a plot basis the latter part of August and the middle of September.

The ratings on 95 inbred lines, grown both in 1942 and 1944, are shown in table 1. In 1944 the inbred lines were divided into two groups, those of Corn-Belt maturity (about 125 to 145 days) being grown in one series and the later-maturing lines in another. The ratings show much less infection

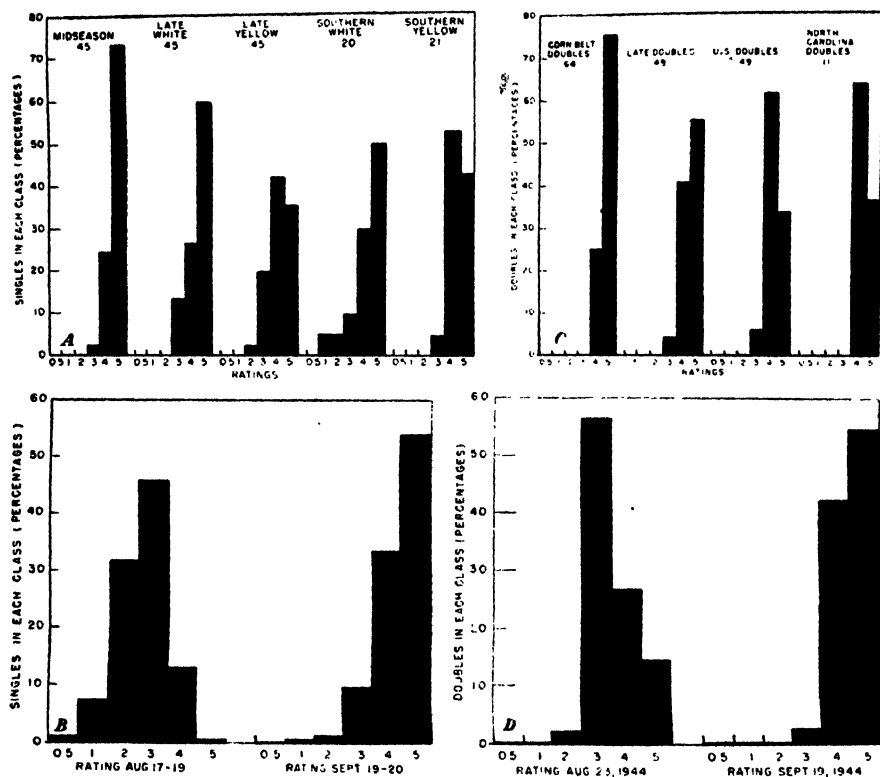


FIG. 2. Frequency distributions of ratings of *Helminthosporium turcicum* leaf blight in single and double crosses of dent corn at Beltsville, Md., 1944. A, 176 single crosses rated September 19-20; B, same single crosses as in A, rated August 17-19 and September 19-20; C, 173 double crosses rated September 19; D, same double crosses as in C, rated August 23 and September 19.

in 1942 than in 1944, but on the whole there is reasonable agreement between the results obtained in the two seasons, certain lines having low ratings in both years and others high. There is, however, no perfect correlation. K64 and Tx155A have just as high ratings in 1942 as in 1944, whereas Ky27, K60, Oh56 with relatively low ratings in 1942 had very high ratings in 1944. Whether these discrepancies are due to differences between strains of the lines used in the 2 years or are due to differences in races of the fungus or to other causes remains to be determined. Only 3 of the lines of Corn-Belt maturity tested, and only 8 of the late inbred lines had light infection

TABLE 1.—*Helminthosporium turcicum* leaf blight ratings on inbred lines of corn in 1942 and 1944 at Beltsville, Maryland

Lines of Corn-Belt maturity	Ratings			Late inbred lines	Ratings		
	1942	1944			1942	1944	
	Aug. 12	Aug. 25	Sept. 16		Aug. 12	Aug. 24	Sept. 15
Tx206	0.5	2.5	2.8	NC34	0.5	0.5	0.6
Tx155A	3.0	3.0	3.0	CI.23	0.5	0.9	1.1
Tx4R3	0.5	3.0	3.8	K175	0.5	0.9	1.5
Tx203	3.0	3.0	3.8	T49B	0.5	1.5	2.3
KB397	1.0	3.0	4.0	T105B	0.5	1.8	2.5
90	3.0	3.8	4.0	K155	0.5	2.0	2.5
Tx61M	1.0	4.0	4.0	T61	0.5	1.8	3.0
L317	0.5	3.3	4.3	T7-6A	0.5	2.0	3.0
B2	1.0	4.0	4.5	T18	1.0	2.8	3.3
CI.4-8	1.0	4.0	5.0	K54	1.0	3.0	3.3
K	1.0	4.0	5.0	Ky122	0.5	2.0	3.5
WF9	2.0	4.0	5.0	K41	0.5	3.0	3.8
Bl 345	2.0	4.0	5.0	Ky58	0.5	3.0	3.8
Pr	2.0	4.0	5.0	T13	0.5	2.0	4.0
Oh56	0.5	4.0	5.0	Kys	1.0	2.8	4.0
461-3	0.5	4.3	5.0	CI.43	0.5	3.0	4.0
38-11	3.0	4.8	5.0	K64	4.0	3.0	4.0
33-16	1.0	4.8	5.0	K148	0.5	3.0	4.0
Oh07	2.0	4.8	5.0	K158	1.0	3.0	4.0
Oh51	1.0	4.8	5.0	K4	1.0	3.3	4.0
CI.187-2	2.0	5.0	5.0	CI.5	1.0	3.5	4.0
CI.540	2.0	5.0	5.0	K166	2.0	4.0	4.0
A	3.0	5.0	5.0	CI.24	1.0	3.0	4.3
Hy	2.0	5.0	5.0	CI.63	0.5	3.5	4.3
M14	2.0	5.0	5.0	T85	0.5	4.0	4.3
R4	3.0	5.0	5.0	CI.21	1.0	4.0	4.5
5120	3.0	5.0	5.0	Ky39	0.5	3.3	4.8
P8	3.0	5.0	5.0	CI.41	3.0	4.0	4.8
Tr	4.0	5.0	5.0	Ky27	0.5	4.0	4.8
66	2.0	5.0	5.0	MoG	1.0	4.0	4.8
Bl 349	2.0	5.0	5.0	CI.2	1.0	3.5	5.0
Mc401	4.0	5.0	5.0	Ky17	1.0	3.8	5.0
Os420	3.0	5.0	5.0	CI.27	1.0	4.0	5.0
ITE701	5.0	5.0	5.0	CI.7	2.0	4.0	5.0
Oh01	3.0	5.0	5.0	K10	3.0	4.0	5.0
Oh67	3.0	5.0	5.0	K180	1.0	4.0	5.0
Oh84	2.0	5.0	5.0	Ky41	3.0	4.0	5.0
Tx127C	4.0	5.0	5.0	Mo940	0.5	4.0	5.0
				T7-2E	0.5	4.0	5.0
				K18	1.5	4.3	5.0
				Ky49	2.0	4.3	5.0
				Mo7Ra	1.0	4.3	5.0
				CI.1	1.0	4.5	5.0
				K124	3.0	4.5	5.0
				Ky13	1.0	4.5	5.0
				Ky21	2.0	4.5	5.0
				T21A	2.0	4.5	5.0
				K17	1.0	4.8	5.0
				K159	3.0	4.8	5.0
				Ky56	1.0	4.8	5.0
				CI.3	3.0	5.0	5.0
				CI.6	4.0	5.0	5.0
				K60	2.0	5.0	5.0
				K124	3.0	5.0	5.0
				K151	2.0	5.0	5.0
				Ky132	3.0	5.0	5.0
				T14	3.0	5.0	5.0

or less in 1944. Of these, one of the lines of Corn-Belt maturity and six of the late lines are listed in table 1. The other four, namely, CI.15, CI.19, Ky114, and Mo21A, were not tested in 1942. All other lines had moderate to very heavy infection. There was less infection in the late lines in both years than in the Corn-Belt lines.

Five groups of single crosses, designated as midseason, late white, late yellow, southern white, and southern yellow crosses, were included in the 1944 experiments. The first three groups each contained all of the 45 possible combinations among 10 parent lines and the last two each contained all but one and all, respectively, of the 21 possible combinations among seven parent lines. Frequency distributions of the ratings for the single crosses in each of the different groups are shown in figure 2, A. In September the midseason single crosses had more very heavy infection than the late and the southern single crosses. The southern white singles had 5 per cent very light and 5 per cent light infection. The late yellow singles are the only others with any light infection. Frequency distributions of the ratings for all 176 singles in August and in September are shown in figure 2, B. Eighty-seven per cent of all single crosses tested in 1944 were heavily infected in September.

The 1944 leaf blight ratings of inbred parents of each group of single crosses and the mean ratings of the single crosses involving each parent line are reported in table 2. K155 was the most resistant of any of the parents of the midseason group and the mean August and September ratings of the single crosses involving this line also were lower than those for any other parent line. The low September rating of Mo22 and the low September average rating for the crosses involving this line indicate that it is probably the most resistant line in the late white group. In the late yellow group K155 again was the most resistant parent line both when judged on its ratings as an inbred line and on the average ratings of the single crosses involving it.

In the southern white group NC34 was the most resistant parent line and the crosses involving it had the lowest average ratings. NC34 had the lowest rating of all the 200 inbred lines. It was significantly more resistant than any other line tested. In the southern yellow group there were no marked differences.

An attempt was made in 1944 to test as many double crosses as were available. The double crosses tested were divided into four groups and these were designated as Corn-Belt doubles, late doubles, U. S. doubles, and North Carolina doubles, respectively. Frequency distributions of the ratings of these double crosses are given in figure 2, C. There was no light infection in any of the double crosses in the September rating, and only 4 per cent moderate infection in the late doubles and 6 per cent in the U. S. doubles in September. The Corn-Belt doubles and the North Carolina doubles were 100 per cent heavily infected, the late doubles and the U. S. doubles were 96 per cent and 94 per cent heavily infected, respectively.

TABLE 2.—*Summary of leaf blight ratings of inbred lines and the mean ratings of their single crosses following inoculation with Helminthosporium turcicum in 1944 at Beltsville, Maryland*

Inbred lines	Ratings Aug. 17-19		Ratings Sept. 19-20	
	Means of crosses ^a	Parent line ^b	Means of crosses ^a	Parent line ^b
<i>Midseason group</i>				
Hy	3.1	5.0	4.7	5.0
L317	2.6	3.3	4.7	4.3
WF9	3.1	4.0	4.9	5.0
38-11	3.1	4.8	4.7	5.0
B3	3.1		5.0	
461-3	2.9	4.3	4.9	5.0
K155	1.8	2.0	4.0	2.5
N6	2.3		4.7	
Oh28	3.0		4.6	
CL7	3.3	4.2	4.9	5.0
<i>Late white group</i>				
33-16	3.0	4.8	4.4	5.0
Ky27	2.1	4.0	4.1	4.8
K55	2.8		4.9	
K64	2.7	3.0	4.4	4.0
H25	2.6	4.3	4.6	5.0
H24	2.7	4.5	4.7	4.8
R30	3.1		4.8	
Mo22	1.8	3.0	3.4	3.0
Tx4R3	1.9	3.0	4.3	3.8
T18	1.6	2.8	4.2	3.3
<i>Late yellow group</i>				
38-11	3.0	4.8	4.3	5.0
H7	2.4		4.5	
K223A	3.2		4.8	
K4	2.3	3.3	4.0	4.0
Kys	2.4	2.8	4.1	4.0
K155	1.8	2.0	3.3	2.5
Oh04	2.9	4.0	4.1	5.0
T8	2.1	3.0	3.4	3.5
CL5	2.3	3.5	3.9	4.0
CL7	3.2	4.0	4.6	5.0
<i>Southern white group</i>				
L13	3.2		4.3	
NC34	1.3	0.5	3.1	0.6
NC37	2.7	4.0	4.6	5.0
R7	3.9		4.9	
T10	2.5	4.5	4.3	5.0
T61	2.1	1.8	3.8	3.0
Tx155A	2.9	3.0	4.2	3.0
<i>Southern yellow group</i>				
R34	2.7		4.5	
R40	3.2	4.0	4.5	4.0
Kys	2.5	2.8	4.0	4.0
K4	2.7	3.3	4.0	4.0
NC12	2.5	3.0	4.2	4.0
CL7	3.2	4.0	4.9	5.0
CL21	3.2	4.0	4.5	4.8

^a Each rating in these columns is the average of the ratings on the single crosses produced by crossing each inbred line listed on the left with all of the other lines of the same group.

^b Ratings on the parental inbred lines themselves.

Frequency distributions of the August and September ratings of 173 double crosses are given in figure 2, D. There was 2.3 per cent light infection in August but none in September; 56 per cent had moderate infection in August with only 2.9 per cent in September; 41 per cent had heavy infection in August, and 97 per cent were heavily infected in September.

The results of these inoculations indicate that there are resistant lines that tend to transmit their resistance to crosses, that earlier lines and crosses are more susceptible than later lines and crosses, and that the double crosses now being used in hybrid corn production are susceptible to leaf blight. NC34 was outstanding in its resistance to *Helminthosporium turcicum* leaf blight and in transmitting this resistance to its hybrids. It is possible that differences in genetic composition of the host, differences in virulence of the fungus, or other factors may complicate the problem of resistance to this disease. Abundance of moisture appears to determine the stage in the development of the plant when the leaf blight becomes severe and the resulting damage to the crop. If the disease develops early in the season when corn plants are only partly grown, the entire crop may be destroyed. If heavy infection develops late in the season, after the ears are well formed and the plants are approaching maturity, losses may be restricted largely to the fodder or may affect both the yields and quality of fodder and of grain.

SUMMARY

Field inoculation experiments were conducted at Beltsville, Md., in 1944 to determine relative susceptibility of inbred lines and crosses of dent corn to *Helminthosporium turcicum* leaf blight. Results of these inoculations were compared with records of leaf blight during the epidemic of 1942.

About 5 acres of corn were inoculated twice weekly beginning the last of June and ending as the plants came into tassel. The disease spread rapidly during August and September.

Leaf blight ratings on 200 inbred lines, 176 single crosses, and 184 double crosses indicate that most lines and crosses are susceptible. NC34 was significantly more resistant than any other line tested. CI.23, K175, Ky114, Mo21A, T49B, T105B, K155, CI.15, CI.16, and Tx206 had only traces to light infection. The other lines tested showed moderate to very heavy infection. In general, the resistance of resistant lines was transmitted to their hybrids.

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A METHOD FOR THE MACROSCOPIC STUDY OF ROOT DISEASES

CORA LEE TERRY GOTT AND G. W. GOLDSMITH¹

(Accepted for publication April 30, 1946)

Pirone² described a method suitable for the commercial propagation of plants from cuttings without use of soil or of any type of solid medium. A modification of this method seemed to offer possibilities in plant pathology, particularly in the study of root diseases, enabling all parts of the plant to be examined macroscopically at any time during the period of infection. The method has unlimited possibilities in permitting the use of different types of culture media to determine parasitic relationships.

METHOD

Seeds of cotton and corn were germinated under sterile conditions on moist filter paper, and when the hypocotyls had emerged to a length of 2 to 3 centimeters, they were placed in root containers similar to those described by Pirone (Fig. 1). Each container consisted of a metal frame

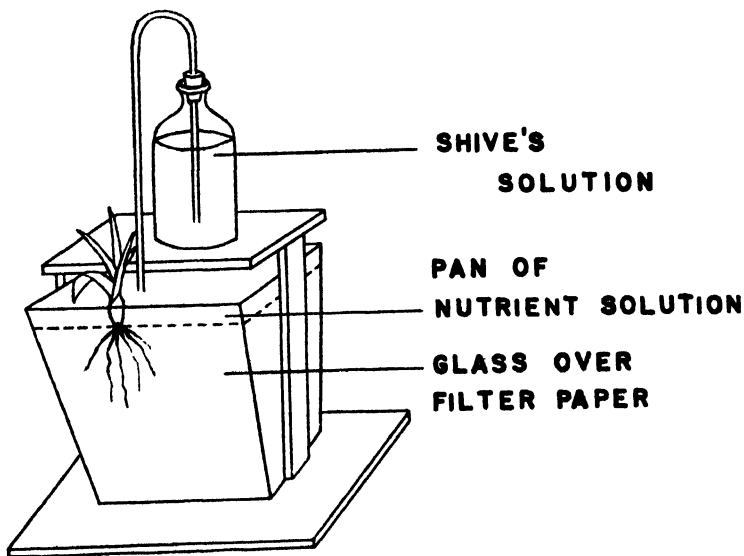


FIGURE 1. ROOT CONTAINER

supporting a glass surface 16 by 24 inches. At the top of the metal frame, supported partially by a wooden frame and partially by the edges of the metal frame, was a metal pan containing nutrient solution. Adjacent to the glass was a strip of filter paper which folded over into the pan containing the mineral solution. The germinating seeds were placed at the top of the

¹ Dr. Goldsmith was Professor of Botany at the University of Texas prior to his death in October, 1943.

² Pirone, P. P. A new method of plant propagation. *Science* 94: 74. 1941.

container between the glass and the filter paper. A black, metal sheet covered the glass portion to prevent light injury to the roots.

The nutrient supplied was Shive's solution³ consisting of 3 salts, potassium dihydrogen phosphate (0.018 M), calcium nitrate (0.0052 M), and magnesium sulphate (0.015 M).

The cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, was used as the parasite in all of the experiments. Corn, which is resistant to this fungus in the field, and cotton, which is susceptible, were used as the host plants. The fungus cultures used for inoculation were grown by Dunlap's⁴ method in order to insure pathogenicity.

After the plants had grown sufficiently to develop a root system approximately 6 inches long, one series each of cotton and corn was inoculated with fresh sclerotia of the fungus. Another series of each was used as a control.

To determine the possibilities of the root-growing apparatus as a method of testing the effects of controlled nutrient solutions on parasitism, preliminary work was done by substituting Na_2SO_4 for MgSO_4 in the Shive's solution, thus creating a magnesium deficiency. Young corn seedlings were placed in the container after 12 hours and were inoculated in the usual manner. They were given the complete Shive's solution for 60 hours and the magnesium-free solution throughout the remainder of the experiment.

RESULTS

The plants were examined daily for symptoms of wilting, and measurements of growth increments of the roots were made whenever practical. Usually this was not possible with cotton roots as they grew too slowly to

TABLE 1.—Measurements on corn plants grown in root containers. Five plants were inoculated with *Phymatotrichum omnivorum* and five were not inoculated

Measurements ^a	Inoculated plants	Noninoculated plants
Epicotyl: length	27 cm.	30 cm.
green weight	10.3 gm.	29.5 gm.
dry weight	1.3 gm.	3.6 gm.
Hypocotyl: length	27 cm.	43.5 cm.
green weight	1.6 gm.	4.2 gm.
dry weight	.45 gm.	.78 gm.
Number of roots	9	13.5

^a Each measurement is the average for 5 plants.

permit accurate measurements. In general, it was found that corn, which is normally immune from the root-rot fungus in the field, could be attacked but not killed under the conditions of the experiment. Plants which were inoculated were usually retarded in growth and lacked the vigor of the control plants. The measurements are in table 1.

³ Shive, J. W. A three-salt nutrient solution for plants. Amer. Jour. Bot. 2: 157-160. 1915.

⁴ Dunlap, A. A. A convenient soil-culture method for obtaining sclerotia of the cotton root rot fungus. Amer. Jour. Bot. 28: 945-947. 1941.

FIGURE 2.
AVERAGE GROWTH OF NORMAL AND INFECTED CORN ROOTS IN SHIVE'S SOLUTION

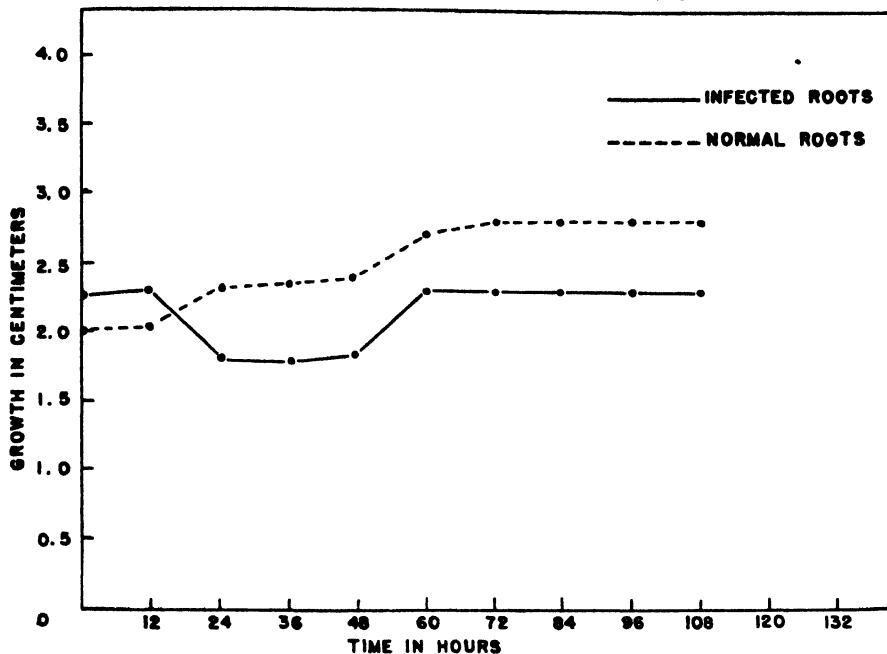


FIGURE 3.
GROWTH OF INFECTED CORN ROOTS IN SHIVE'S SOLUTION

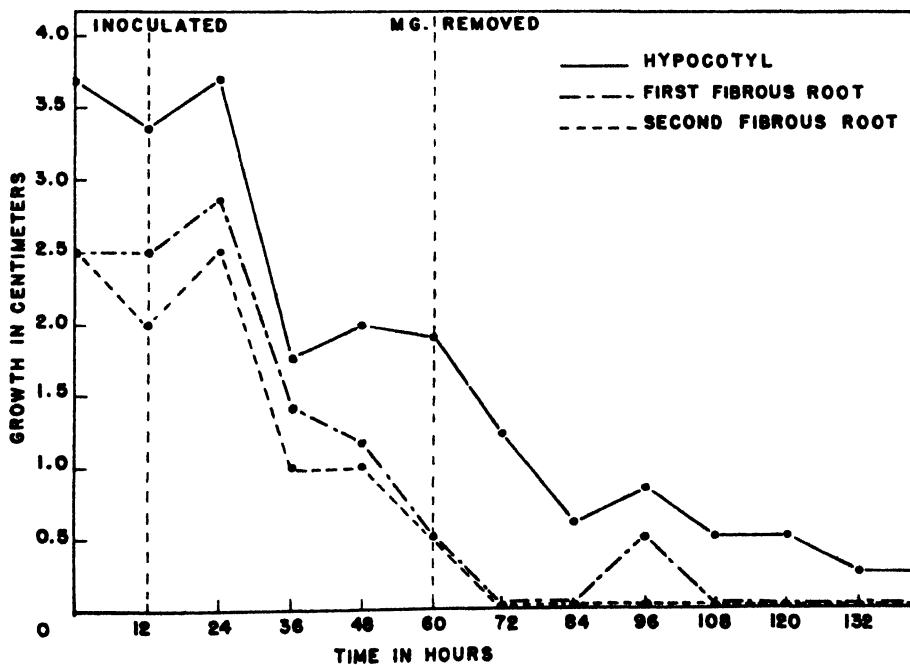


Figure 2 shows the effect of inoculation on the root growth of 5 corn plants as well as the growth of a similar number noninoculated. Measurements are in centimeters and were made at 12-hour intervals. After 36 hours the root-rot fungus caused a constantly diminishing growth rate of the corn roots, although none of the plants was killed during the course of the experiments. In contrast to this, the control plants continued to grow normally.

Cotton seedlings, which are usually immune⁵ in the field, gave results similar to those of corn when grown under experimental conditions. The controls continued to grow throughout the experiment while inoculated seedlings showed symptoms of root necrosis very early. The inoculated roots ceased growing 24 hours after inoculation and soon decayed. If there were not other secondary roots to serve as sources of nourishment, the entire plant soon died. Sections through the infected areas showed the root-rot fungus in the cells of the cortex, endodermis, and xylem.

The corn plants which were deprived of magnesium supply 60 hours after inoculation showed even more symptoms than those which were infected in the presence of full nutritional requirements. Growth rates continued diminishing, and after 3½ days root growth ceased. Figure 3 shows the measurements of 3 typical roots during the experiment. Within a week following the cessation of growth, the plants wilted and died.

SUMMARY

1. A macroscopical method for the examination of root infections has been devised.
2. Cotton seedlings growing in root containers were killed after inoculation with *Phymatotrichum omnivorum*.
3. Corn, which is immune from *Phymatotrichum omnivorum* in the field, was rendered susceptible to attack but not killed.
4. Corn seedlings can be rendered susceptible to typical infection by *Phymatotrichum omnivorum*, which results in death, by withholding magnesium from the nutrient solution.

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⁵ Blank, L. M. The susceptibility of cotton seedlings to *Phymatotrichum omnivorum*. *Phytopath.* 30: 1033-1041. 1940.

GREEN DWARF: A VIRUS DISEASE OF POTATO¹

J. A. MILBREATH²

(Accepted for publication May 17, 1946)

Green dwarf, an undescribed disease of potatoes, has been present in Oregon for a number of years. The disease is caused by a virus which seems to be distinct from all other viruses which have been reported on potatoes. Green dwarf has been collected from Baker, Crook, Deschutes, and Malheur Counties in Oregon. Since it is often found in fields of potatoes being grown for seed, it is probable that the disease is more widely distributed but not recognized. Tuber-perpetuated green dwarf was found in a number of fields of Netted Gem potatoes grown from seed which had been produced in Montana.

SYMPTOMS OF THE DISEASE

Tuber perpetuated. The outstanding symptoms of tuber-perpetuated green dwarf are late emergence and extreme dwarfing. It is not uncommon for a mature green-dwarf potato plant to be only six inches high, while adjacent healthy plants are two feet or more high. The basal leaves appear normal, but are close together in a basal rosette of four or five leaves. The remaining terminal growth is dwarfed and malformed, and the growing point is pinched together in a cluster of small leaves (Fig. 1, A). The leaflets on the young leaves cup upward, forming small boat-like structures (Fig. 1, C). The plants are normal green or sometimes even darker green than normal. The tubers are small, but have no other symptom.

The tuber-perpetuated symptoms of green dwarf in the greenhouse are quite different from those of field-grown plants. Emergence is one to two weeks later than normal and when the sprout finally breaks through the soil a dark green, leafy bud develops, which does not unfold its leaves for some time. The entire plant may not be over one to two inches tall, two or three weeks after emergence (Fig. 2). Gradually the stem elongates and the older leaves expand into small dark green, unmottled structures, often not over one to two inches long. The plants are very stiff and erect, and the growing point remains compact or pinched together. After two months of growth, Green Mountain plants from tubers inoculated the previous year with green dwarf virus, produced blossom clusters on plants not over six inches tall, and the leaf spread of the plants did not exceed two inches (Fig. 1, B). Some plants, especially the White Rose variety, after a late emergence and slow development, will begin to grow normally and produce a plant 12-18 inches tall. These plants appear normal, except that leaves are short and leaflets small, which gives the entire plant a narrow, leggy appearance.

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² The assistance of F. P. McWhorter and H. H. Millsap in the photographic work is gratefully acknowledged.



FIG. 1. A. Terminal growth of a field-grown Netted Gem potato plant affected with green dwarf. The symptoms are much the same for current-season and tuber-perpetuated green dwarf. B. A mature greenhouse-grown plant of Green Mountain from a tuber infected the previous year with green dwarf. C. Typical leaf symptoms of green dwarf on field-grown Netted Gems.

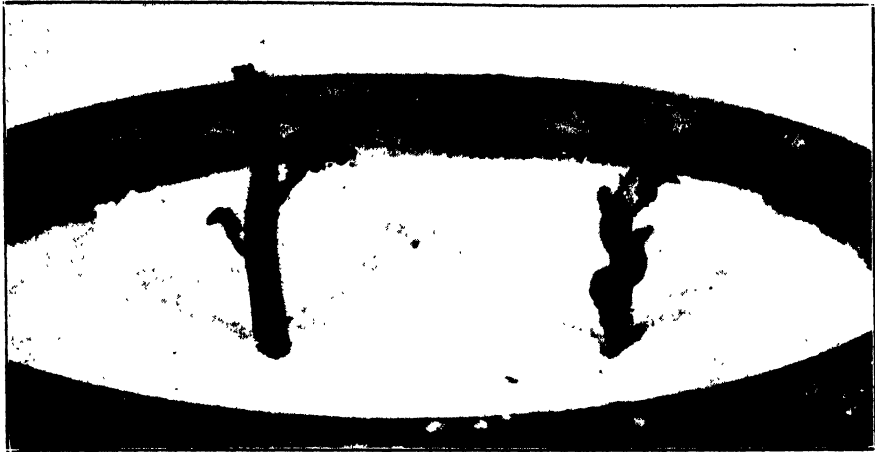


FIG. 2. Tuber-perpetuated symptoms of green dwarf. Photographed three weeks after emergence. All varieties studied emerge late and grow slowly after emergence.

Current season symptoms. The current season infection of green dwarf from natural spread causes very little injury to the plants. The symptoms are usually limited to the terminal growth of one or more lateral branches, and are very similar to the symptoms of the terminal growth of tuber-perpetuated green dwarf (Fig. 1, A). The growth stops or becomes very much dwarfed. The leaves are small and the leaflets are misshapened and cup upward (Fig. 1, C). The leaves of the growing point are clustered together or pinched and dwarfed.

No current season symptoms have been produced in the greenhouse by inoculation.

TRANSMISSION OF THE DISEASE

The disease has not been transmitted by the carborundum or the tuber core graft methods of inoculation. It is readily transmitted by grafting a portion of diseased stem onto a healthy plant. Green dwarf has been trans-

TABLE 1.—*Results of inoculating potato plants with the green-dwarf virus*

Variety	Method of inoculation	No. of plants inoculated*	No. of plants with green dwarf
Netted Gems	Carborundum	10	0
White Rose ..	do	5	0
Burbank	do	5	0
Netted Gems	Core graft	17	0
Netted Gems	Side graft	8	7
White Rose ..	do	9	2
Burbank	do	3	2
Green Mountain	do	4	4
X-immune seedling	do	5	1

* A similar number of check plants were grown for each inoculation. Tubers were saved from these plants and were again used as checks for tuber perpetuated symptoms. None of the checks developed green dwarf.

mitted by grafting infected Netted Gems to healthy Netted Gems, Burbank, White Rose, Green Mountain, and X-immune seedling. Table 1 gives the details of these inoculations.

The virus apparently moves slowly through the plant because tubers taken from plants with current season symptoms may not all produce green dwarf. Likewise, individual tubers cut in several pieces and planted as a tuber unit may produce only one green-dwarf plant in the unit.

LOSSES FROM THE DISEASE

The disease has not developed to the point where it causes serious losses. Most fields have less than 1 per cent of the disease. In some areas there is considerable current season spread of green dwarf, and the disease is a constant worry to growers of certified seed in these areas.

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PHYTOPATHOLOGICAL NOTES

Transmission of Peach Wart by Graft Inoculations with Affected Fruit Tissue.—On August 24, 1942, three J. H. Hale and three Elberta peach trees in the plots at Moscow, Idaho, were inoculated with fruit tissue affected with peach wart. Wedges of warty tissue approximately 35 mm. long, 3 mm. thick, and 4 or 5 mm. wide at the large end were placed in T cuts in the bark of the trunks of young peach trees. Three pieces of tissue were set on each tree and held in place with rubber budding strips. The external surface of the fruit was placed outermost and since the warty tissue was either very tough and leathery or woody there was very close contact between the wedges and the stock under the rubber band pressure. Observation showed that in several cases the peach fruit tissue retained its normal appearance and seemed alive for at least a month. Cochran and Rue¹ secured transmission of peach mosaic by a similar grafting technique using leaf, fruit, bark, and wood tissue. They state that in the case of leaf tissue it appeared to have united with the wound callus.

In August, 1945, examination showed that transmission had occurred in all cases inoculated by the fruit tissue method. No control inoculations were made with tissue wedges of healthy fruit but the trees inoculated by this technique or by bud inoculation were the only ones showing warty fruit in the plots which contained several hundred trees. The virus causing peach wart is known to spread in an orchard² but even in older plantings the spread is slow. The experience with peach wart and the experimental evidence presented indicate that the presence of the disease on these trees was not due to contamination but to actual transmission of the virus. Fruit symptoms were typical and severe.² It is not known whether tissue union occurred; according to Dorsey and McMunn³ it is generally agreed that in nearly mature peach fruits there is no meristem present in flesh tissue. There remains the possibility, however, that in peach fruits affected by wart, some meristematic tissue may exist long after it has disappeared in normal fruits. Transmission by this fruit tissue method and variations of it offer a useful technique for studying properties of the peach wart, mosaic, and perhaps other viruses.—EARLE C. BLODGETT, Formerly Assoc. Plant Pathologist, Idaho Agricultural Experiment Station, Moscow, Idaho.

Stubborn Disease of Citrus, a Virosis.—At the time the history and the description of the stubborn disease on navel oranges was first reported,¹ it was suspected that it might be a virus disease rather than a genetic variation. The experimental evidence developed since that time together with further

¹ Cochran, I. C., and John L. Rue. Some host-tissue relationships of the peach mosaic virus. (Abstr.) *Phytopath.* 34: 934. 1944.

² Blodgett, Earle C. Peach wart. *Phytopath.* 33: 21-32. 1943.

³ Dorsey, M. J., and R. L. McMunn. Tree-conditioning the peach crop. *Ill. Agr. Exp. Sta. Bul.* 507. p. 353-357. 1944.

¹ Fawcett, H. S., J. C. Perry, and J. C. Johnston. The stubborn disease of Citrus. *California Citrograph* 29: 146, 147. 1944.

observations has led to the definite conclusion that stubborn disease on navel orange trees is of virus origin.

The symptoms on navel oranges when pronounced consist of a brush-like growth of twigs, due to abnormal branching from multiple buds. The small branches often bend down over most of their length and turn up at the outer ends. The leaves tend to be broader and shorter and bend upward more on each side of the midrib than those of healthy trees. The foliage, especially on the south side, usually consists of an untimely autumn growth which becomes somewhat chlorotic. Some of the fruit develops abnormally into forms that resemble an acorn shape^{1, 2} with the rind of the stem half or portions of it remaining normal in thickness while the rest of the rind decreases abruptly in thickness with the thinnest portion near the styler end. In severely affected fruit the pulp below the thin portions of the rind has a sour or bitter taste and disagreeable odor. Trees tend to become unproductive and are often mistaken for the so-called "Australian type" of navel.

The most definite experimental evidence was obtained by budding sweet orange seedlings with buds from trees with stubborn disease (March, 1939) and topworking the resulting trees with buds from a healthy navel orange tree (May, 1943).¹ At the present time (March, 1946) the growth from the originally healthy navel buds has not only developed the typical branch and leaf symptoms, but some of the fruits have the "acorn" symptoms.^{1, 2}

Healthy buds from this same source placed on trees at the same time, not showing stubborn symptoms, grew out and produced normal branches, leaves, and fruit.

The symptoms on the diseased trees developed slowly. This experiment corroborates many previous observations by propagators and growers that trees with these symptoms, when top worked with carefully selected healthy navel orange buds or scions, again had the same disease symptoms after developing a new top.

There has not been time as yet to determine definitely whether or not the so-called "crazy top" and "acorn" or "pink nose"³ of grapefruit is the same disease although the symptoms are similar. Transmission experiments on grapefruit and other varieties were begun in 1944.

To summarize the results: Typical symptoms have been induced in tops of navel orange trees grown from healthy buds placed in diseased trees. Tops grown from healthy buds from the same source placed in healthy trees remained healthy.

The virus causing the stubborn disease may be designated and described as follows:

Citriwir pertinaciae⁴ (pertinaciae, genetive of pertinacia = pertaining to stubbornness), the virus causing stubborn disease of citrus. Induces on

² Haas, A. R. C., L. J. Klotz, and J. C. Johnston. Acorn disease in oranges. California Citrograph 29: 148, 168-169. 1944.

³ Burgess, P. S. Agricultural Chemistry and Soils: Boron. Arizona Agricultural Experiment Station Rept. 52: 7. 1942.

⁴ Fawcett, H. S. Citrus viruses. Phytopath. 31: 356-357. 1941.

navel orange trees multiple buds and abnormal branchings resulting in a brush-like growth. Induces broader and shorter leaves than normal and untimely, somewhat chlorotic, growth in the autumn. Causes some of the fruit to take on an "acorn" shape in which the rind at the stem end is normal, becoming abruptly thinner and smoother over the remainder of the surface. Mature fruits tend to be sour and bitter at the navel end.—H. S. FAWCETT, Citrus Experiment Station, Riverside, California.

Experiments on the Overwintering in the Soil of Bacteria Causing Leaf and Pod Spots of Snap and Lima Beans.—The overwintering in nonsterile soil of bacterial pathogens of snap and lima beans (*Phaseolus vulgaris* and *P. lunatus* var. *macrocarpus* (Benth.) Van Eselt.) is still an open question, although in the case of *Xanthomonas phaseoli* (E. F. Sm.) Dowson there is good circumstantial field evidence for survival through the winter in bean plants thrown on compost heaps. The writer has made the following experiments:

Idaho-grown snap bean seed of the Bountiful variety was planted in May, June, and July at the Arlington Experimental Farm in Virginia, in a plot where snap beans heavily infected with *Pseudomonas medicaginis* var. *phaseolicola* (Burkh.) Stapp and Kotte had been plowed under the previous autumn. No infection ensued.

The soil hold-over of *Pseudomonas medicaginis* var. *phaseolicola* and *Xanthomonas phaseoli* was tested by burying in pots of Arlington Farm soil lima bean leaves and pods with fresh autumn infections of these organisms. The pots were sunk in the ground out of doors. The following spring Full Measure snap bean plants from Idaho seed were grown therein and kept well watered, but no signs of infection appeared on them.

A similar experiment on the Eastern Shore of Maryland likewise produced negative results. In this case the buried lima bean (King of the Garden variety) leaves bore fresh autumn infections of *Pseudomonas syringae* van Hall and the varieties planted in this soil the following spring were King of the Garden lima bean and Idaho-grown Bountiful snap bean.

Inoculation experiments to test further the overwintering of bean bacterial pathogens were made as follows:

Lima bean leaves with autumn infections of *Pseudomonas syringae* had been exposed to weathering from October 22 till June 7, but had not been in contact with the soil. On the latter date they were crushed to powder and dusted on sprayed pricked seedlings of Ideal pole lima in a damp chamber. The plants were sprayed again after dusting and kept 3 days in the damp chamber. No *Pseudomonas syringae* appeared on any of the 19 plants, but *Xanthomonas phaseoli* was isolated from water-soaked spots on inoculated leaves of 3 of them. The check plants had no sign of infection. Had the *Xanthomonas phaseoli* been associated with *Ps. syringae* in the lesions on the weathered leaves? *X. phaseoli* has been found now and then, in small numbers, in lesions caused by various other bean bacterial pathogens.

The preceding experiment was repeated a week later, but with a water suspension of the powdered dried leaves. No infection resulted.

Soil in which lima bean leaves with fresh autumn infections of *Pseudomonas medicaginis* var. *phaseolicola* had been buried out of doors on October 22, was used as inoculum on June 7. It was applied to the leaves of Ideal pole lima seedlings in a damp chamber, after which the plants were sprayed and pricked. No infection resulted.—FLORENCE HEDGES, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, Plant Industry Station, Beltsville, Maryland.

REPORT OF THE 1946 ANNUAL MEETING OF THE NEW ENGLAND DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

In order to coincide with the New England-New York Spray Specialists' Conference, the 1946 annual meeting of the New England Division was held on December 4, 5, 6, 1945. Members of A.P.S. in New York, New Jersey, and Pennsylvania were invited to participate in these meetings. Of the 75 persons who registered, approximately half were from the invited states. In addition to the 14 papers presented, 3 symposia were conducted: Potato Diseases, Seed Treatments, and Teaching Plant Pathology and Trends in Research.

Officers of the New England Division for 1946 are: President, Frank L. Howard, Rhode Island State College, Kingston; Vice President, P. J. Anderson, Connecticut Experiment Station, Windsor; Secretary-Treasurer, Thomas Sproston, Jr., Massachusetts State College, Amherst; Councilor, M. C. Richards, University of New Hampshire, Durham.

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

The Fungi of Pole-rot of Tobacco. ANDERSON, P. J. A considerable number of species of bacteria and fungi, *Alternaria*, *Macrosporium*, *Fusarium*, *Penicillium*, *Aspergillus*, *Cladosporium*, and others, are readily isolated from pole-rot damaged leaves of tobacco in the curing shed. When leaves are inoculated these species are found to be either nonpathogenic or so weakly pathogenic that they could not be responsible for destructive epidemics. Two other fungi, *Sclerotinia sclerotiorum* and *Botrytis cinerea*, rarely isolated from old lesions, are always isolated from the very young lesions, are highly pathogenic on tobacco leaves, and are responsible for the severe epidemics in New England. They are most active on leaves that are still green. Later these two fungi are destroyed by the other organisms mentioned. Pole-rot starts on tobacco while it is still standing in the field.

A Laboratory Assay for Stability of Organic Fungicide Residues. BARRATT, R. W. Laboratory assays have been designed to measure the following factors causing failure of fungicides in the field: tenacity, previous material in the spray tank or additives, decomposition during application, and decomposition of the spray residue from ultraviolet light, leaf exudates, atmospheric gases, rain water, and soil on leaves. The rate of decomposition is an important statistic. Essentially, the technique involves the spraying of coated glass slides in a dosage series employing a dose ratio of 2 or $\sqrt{2}$. Replications of each concentration are sprayed, one set being assayed immediately for fungicidal potency. The remaining slides are stored at a constant temperature in a closed chamber having the environment being studied. By assaying slides stored for different periods of time, the LD90 in terms of micrograms per sq. in. can be determined. From the shape and position of the curves resulting from charting the logarithm of the LD90 against the time of storage in days, the relative rates of decomposition of the fungicides can be determined. By using this technique the rate of decomposition of disodium ethylene bis dithiocarbamate in the presence of water and oxygen has been studied and can be slowed down by the addition of zinc sulphate and lime.

Control of Damping-off by a Delay in First Watering After Seeding. DORAN, W. L. When seeds were sowed in soils infested with *Pythium*, the soils having a moisture content of not more than 30 per cent of the water-holding capacity at time of seeding, damping-off, especially pre-emergence damping-off, was much less severe if soil was not watered for the first time until several days after seeding. Numbers of plants which lived in such soil first watered four or five days after seeding were, as compared with numbers which lived in soil watered immediately after seeding, increased by the following percentages: tomato, 39 per cent; eggplant, 27 per cent; pepper, 37 per cent; onion, 96 per cent; lettuce, 89 per cent; beet, 133 per cent; and cabbage, 124 per cent. Stands were not improved by

a delay in first watering if the soil at time of seeding had a much higher moisture content. Stands were usually more improved by a delay of four to five days before the first watering than by a shorter interval. They were usually as much improved by waiting four to five days as by waiting longer and any further delay may be undesirable because of a corresponding delay in emergence. The method is not suggested as a substitute for soil or seed treatment with fungicides but, lacking them, it is a worthwhile cultural practice.

Stimulation and Retardation of Germination of Some Vegetable Seeds Resulting from Treatment with Protective Fungicides. FOSTER, A. A. Copper fungicides injured cabbage, cucumber, and pea seed and stimulated beet, eggplant, pepper, and spinach seed germinated in Petri plates or steamed soil. Nitroprusside tests showed that copper-sensitive seeds contained the sulphydryl radical while others did not. Copper treatment reduced or eliminated sulphydryl groups in seeds. Addition of the amino acid, cysteine, which contains the sulphydryl groups, resuscitated pea seeds that would not germinate following copper treatment. Measurements of oxygen uptake by ground pea and spinach seed showed that pea respiration was retarded by copper but that spinach respiration was not. Pea embryos from treated seed had lower fresh and dry weights than the check. The specificity is interpreted as evidence that the respiratory system of cabbage, cucumber, and pea is catalyzed by enzymes requiring the sulphydryl group while the respiration of beet, eggplant, pepper, and spinach is catalyzed by an enzyme requiring copper. Effects of chloranil and tetramethyl-thiuram-disulphide could be interpreted similarly.

Distinguishing Permeation from Toxicity of Fungicides. HORSEFALL, J. G., R. W. BARRATT, and A. GRIES. Usually, toxicity data do not distinguish between permeation of the chemical into a cell from damage to its contents. Sodium sulphate and other monovalent electrolytes known to increase permeability of cells have been found to increase the potency of disodium ethylene bis dithiocarbamate and *o*-quinone dioxime to *Macrosporium sarcinaeforme*. That this is due to increased permeability and not to increased toxicity is indicated by the fact that the effect can be counteracted by calcium and other divalent electrolytes which are known to reduce the permeability effects of sodium salts. If the toxicant is held constant and sodium sulphate is increased, potency waxes and wanes periodically, but the wavy curve can be rectified by varying concomitantly the amount of calcium. Assuming that the effect is due to variations in permeation through a colloidal gel, in this case the cell wall of the spore, the periodic characteristic resembles the phenomenon in Liesegang rings. Zinc sulphate can synergize disodium ethylene bis dithiocarbamate by liberating sodium sulphate into the medium as the zinc salt is being formed. The toxicity rises with addition of zinc sulphate until all the Na has been replaced, despite the fact that the zinc salt is less potent than the sodium salt. Apparently, the increased permeation more than counterbalances the reduced toxicity of the zinc salt. Calcium in the mixture, however, antidotes much of the effect.

Interactions of Concentration, Pressure, Time, and Orifice in Spraying. HORSEFALL, J. G., NEELY TURNER, and A. D. McDONNELL. The interactions of the factors in spraying were studied by applying variable amounts of material per acre. As the amount of Bordeaux mixture on potatoes or calcium arsenate on beans was increased, the rate of deposit increase was in the following order: concentration > time > pressure > orifice diameter. Deposit increased more rapidly with short than with long spray time, with low than with high pressure, and with small than with large orifices. Using a balanced triangular design with concentration constant, calcium arsenate deposited best on potatoes with high pressure, short time, and small orifices. Deposits, however, may have different tenacity and coverage as a function of technique. The bigger the deposit of calcium arsenate, the faster it washed off in the rain. Applications with short spray times, therefore, washed more rapidly than those from long spray times and this tended to nullify the advantage in original deposition. An attempted assay of coverage was made with the log-probit dosage-response curve using Bordeaux mixture and leafburn on potato. The laboratory conclusion that good coverage should be evinced by a steep slope was not confirmed because of the strong concave curvature which presumably resulted from the fact that the big deposits washed more rapidly than the smaller.

Dutch Elm Disease Studies in Massachusetts During 1945. MCKENZIE, M. A. As of December 1, *Ceratostomella ulmi* (Schwarz) Buisman, the causal fungus of the Dutch elm disease, was isolated from 31 additional trees or wood samples during 1945 in Massachusetts. A total of 74 confirmations by culture for the fungus have been made since 1941: Berkshire County 54, Hampden County 18, Hampshire County (first report) 2. In all locations where the fungus was found during the year detailed surveys were conducted and related beetle-infested bark was burned by property owners, town tree wardens, city foresters, State departments, public utilities, and other cooperating agencies and indi-

viduals. As supplements to this control effort, suggestions were furnished tree owners concerning the general health of elms, spray programs for the control of leaf-feeding insects and particularly for the reduction to a minimum of the elm bark beetle population. Accordingly, the record of attempted control is indicated briefly. In general a few diseased trees have been found in succeeding years after the first afflicted tree was reported for a town, but increase of the disease has not been extensive. In the city of Westfield, the first diseased tree was found in 1942; a second tree was found affected in 1945. In the town of Alford, the first diseased tree was found in 1941; 2 additional ones were found in 1944. In the town of Richmond, 7 trees were confirmed for disease in 1945; apparently some build-up of the fungus occurred there before any diseased trees were found. In urban areas the amount of activity of the citizens is believed to be an important factor in disease increase, while in rural areas storm damage sometimes suddenly creates abundant material suitable for breeding by bark beetles. The increased use of oil as fuel in the postwar period may be an aid to disease control, if storage of elm as fuelwood is thereby reduced.

Apple Leaf Structure in Relation to Penetration by Spray Solutions. PALMITER, D. H., E. A. ROBERTS, and M. D. SOUTHWICK. Spray solutions containing minor elements, nitrogen, "hormones," and organic fungicides have been applied as foliage sprays to apple trees to reduce nutritional deficiencies, prevent fruit drop, or increase resistance to disease. The fact that such applications have given results showed that they had penetrated the leaves and raised the question of how penetration occurred. Standard microchemical technique for differentiating between cutin, cellulose, and pectic substance showed the avenue by which water-soluble materials might pass through the cuticle and surround the epidermal and interior cells. The apple leaf cuticle was not a solid mass of cutin but a laminated tissue composed of discontinuous layers of cutin, cellulose, and pectic materials. The stains showed a continuous avenue of pectinacious substance from the exterior of the cuticle to the epidermal cells and vein extensions. The penetration of apple leaves was demonstrated by placing cut terminals in a 0.5 per cent solution of ferric sulphate and spraying the leaves with a 0.3 per cent solution of sodium dimethyl dithio carbamate. As the sulphate, taken up by the stem, reacted with the carbamate, taken through the cuticle, a black precipitate formed from the cuticle along the veins and into the stem. It was most dense in areas of greatest pectic content.

Effect of Inorganic Fertilizers on Defoliation of New Hampshire Victor Tomatoes by Alternaria solani. RICHARDS, M. C., and R. C. JONES. In earlier investigations, tomato varieties having a heavy fruit to leaf ratio defoliated earlier than those with a high leaf to fruit ratio. The leaves appeared to become susceptible to attack by the pathogen as the nutrients were drained from the leaves by the rapidly developing fruits. In 1944 and again in 1945 attempts were made to supply the roots of the test plants with an excess of N, P, and K so that a higher level of these nutrients could be maintained in the leaves during the fruiting period. The New Hampshire Victor tomato variety was used, as it fruits abundantly and defoliates severely. Inorganic nutrients of N, P, and K were added to the soil at the base of each plant, about 6 inches deep, when the plants were set in the field from 3-inch pots. The tests were arranged in randomized blocks with five replications per treatment. In one block ammonium nitrate, calcium cyanide, ammonium sulphate, sodium nitrate, and Uramon (urea) were used as N sources and applied at the rate of 100 pounds of N per acre plus P_2O_5 , 75 pounds, and K_2O , 45 pounds per acre. In other tests the N-P-K ratios were varied from 0-2-1.75 to 4-2-1.25, the nitrogen varying from 0 to 17, 35, 70, and 140 pounds per acre, with P and K remaining as given above. In further tests the total pounds of N, P, K per acre were varied. In general, there was an increase in the total fruits per plant with increases in nutrients applied. As a result, the high fruit to leaf ratio was maintained, and defoliation was as heavy on these as on the checks. Although abundant soil nutrients were available, a limited number of tissue tests did not reveal increases for N, P, or K in the leaf petioles.

Control of Alternaria Blight on Tomatoes with Fungicides. RICHARDS, M. C., and R. C. JONES. Six applications for each of eighteen fungicide treatments were made during the 1945 season on New Hampshire Victor tomatoes to control *Alternaria solani*. Single hill units with ten replicates for each treatment used were arranged in a randomized block. The concentrations of the materials used and their effect on control of defoliation were as follows: Dithane (disodium ethylene bisdithiocarbamate) (4-100 + $ZnSO_4$ and lime**), Puratized (phenyl mercuri triethanol ammonium lactate) (1-20,000**), Bordeaux (3-3-50**), Zerlate (zinc dimethyldithiocarbamate) (2-100**), Copper oxychloride sulphate (6-100**), Phygon (2,3-dichloro-1,4-naphthoquinone) (1-100**), Fermate Dust (ferric dimethyldithiocarbamate) (10-90*), Tribasic $CuSO_4$ (3-100*), Spergoa (tetrachloro-para-benzoquinone) (1-100*), Fermate + DDT (2-0.6-100), Fermate (2-

100), Fermate (1-100), Fermate Dust + DDT (10-3-87), Bismuth subsalicylate (1½-100), Fermate (1-100) + sticker, Q15 (lauryl isoquinolinium bromide) (½-100), No Fungicide. Average weights in pounds of marketable fruit per plant for each treatment were: Zerlate (8.20**), Puratized (7.93**), Phygon (7.70**), Fermate 2-100 (7.47**), Tribasic CuSO₄ (7.34**), Fermate 1-100 (7.09*), Bismuth subsalicylate (6.30*), Fermate 1-100 + sticker (6.00*), Dithane (6.00*), Fermate Dust (6.00*), Fermate + DDT (5.80), C.O.C.S. (5.67), Bordeaux (5.50), Fermate Dust + DDT (5.30), No Fungicide (5.00), Spergon (4.80), Q15 (3.98). None of the fungicides prevented eventual complete defoliation by *Alternaria*. Definite injury to foliage was noted with Dithane, and stunting of the plants was indicated with Bordeaux and Copper oxychloride sulphate.

** Highly significant (99: 1).

* Significant (19: 1), better than "No Fungicide."

The Incidence of Common Scab on Green Mountain Potatoes in Soils at Different pH Levels. STEINMETZ, F. H. A field which had been in sod for a number of years was fall plowed and laid out into plots to which agricultural lime and finely divided sulphur were applied in order to establish plots with calculated pH levels as follows: 4.5, 5.0, 5.5, 6.0, 6.5. The 4.5 pH series received 2000 pounds of sulphur per acre, the 5.0 pH series received 1000 pounds. The 5.5 pH series was not treated because the soil approximated this pH level. The 6.0 pH series received 2000 pounds of calcium carbonate per acre, while the 6.5 pH series received 4000 pounds. One series of these treated plots received 600 pounds of sulphur per acre. The amendments were applied only once, in the spring before planting the initial potato crop. The potato crop failed the first year on the plots receiving 2000 pounds of sulphur and was suppressed on the plots which received 1000 pounds. After the second potato crop the suppressing effect of sulphur was not marked. The plots which received the 600-pound rate of sulphur produced good yields of scab-free potatoes each year. During the first crop year none of the plots produced scabby potatoes. After 10 years, the increase of scab was in direct relation to the increase in pH level above 5.5. At present the plots with the original pH levels adjusted to 6.0 and 6.5 produce nonmarketable scabby potatoes.

Soil Applications of Oxyquinolin Benzoate for the Control of Foliage Wilting in Elms Caused by Graphium ulmi. STODDARD, E. M. Elm trees, 1.5 inches D.B.H., were treated in lots of 10 trees each with an aqueous solution of oxyquinolin benzoate in concentrations of 0.1, 0.05, 0.025, and 0.0125 per cent at the rate of 10 gallons per tree, applied to the soil. Each concentration was applied as a single application of 10 gallons and 5 applications of 2 gallons each on alternate days for 10 days. Duplicate series of applications were made 10 days before and 10 days after inoculation of the trees with *Graphium ulmi*. A concentration of 0.1 per cent reduced the trees that wilted to 27.5 per cent as compared to 73.4 per cent on the checks. Plots treated with this concentration uniformly had fewest trees wilting and the least wilting per tree, irrespective of manner or time of treatment. The plots treated with a single dose at all concentrations had more trees wilting and less wilting per tree than plots treated with multiple doses. There was no difference between plots treated before and after inoculation, either in number of trees wilting or the amount of wilting per tree. From the fact that *Graphium ulmi* was isolated from an approximately equal number of trees in all the treatments and the checks, it is suggested that the effect of the oxyquinolin benzoate was due to antidoting of the fungus toxin causing the wilting and was not due to fungicidal action.

Glyoxalidine Derivatives as Foliage Fungicides: Laboratory Studies. WELLMAN, R. H., and S. E. A. MCCALLAN. The glyoxalidine or imidazoline nucleus is: $\text{HN}-\text{CH}=\text{N}-\text{CH}_2-\text{CH}_2$. Sixty-five glyoxalidines containing substituents in the 1- or 2-

positions were examined. In laboratory slide-germination tests, maximum fungistatic action is achieved with glyoxalidines having a straight chain substituent containing 13 to 17 carbon atoms in the 2- position. Substituents in the 1- position such as hydroxyethyl, aminoethyl, or butyl do not markedly affect fungistatic action. The various glyoxalidines are removed from solution by spores (or charcoal) in amounts proportional to their fungistatic action. Their action is fungistatic and not fungicidal. Addition of oil or lead arsenate increases fungistatic action in laboratory tests. The 2-heptadecyl glyoxalidine is inherently tenacious in thin films on glass slides. In greenhouse experiments maximum phytotoxicity is reached with the 11 carbon atom side-chain in the 2- position. The ratio, highest concentration giving no plant injury over LD50, for 1-hydroxyethyl 2-undecyl glyoxalidine is 13.5; for 1-hydroxyethyl 2 heptadecyl glyoxalidine it is 1450. Side chain unsaturation increases phytotoxicity as does increasing length of chain in the 1- position. Quaternary-ammonium addition compounds were as fungistatic and were more phytotoxic and less water soluble than parent compounds. These materials were phytotoxic to toma-

toes at concentrations which would not control late blight, and they were moderately effective against snapdragon rust though not phytotoxic.

Glyoxalidine Derivatives as Foliage Fungicides: Field Studies. THURSTON, H. W., JR., and J. B. HARRY. The following compounds: 2-heptadecyl glyoxalidine (C.P.I. No. 341), 1-hydroxyethyl 2-heptadecyl glyoxalidine (C.P.I. No. 337) and 1-aminoethyl 2-heptadecyl glyoxalidine (C.P.I. No. 630) have been studied in the field for five years. Crops and diseases under observation included roses (black spot), potatoes (late blight), apples (scab and rust) and sour cherries (leaf spot). On roses, Nos. 337 and 341 were tested and gave only fair control of black spot. On potatoes, Nos. 337 and 341 were tested and failed to control late blight; the compounds probably were injurious; and potato yields were not equal to those from Bordeaux plots. On apples, all three of the glyoxalidines gave scab control equal to the standard lime-sulphur spray program. The glyoxalidines gave less injury, especially to the foliage, and satisfactory color and finish to the fruit. The 2-heptadecyl glyoxalidine was compatible with lead arsenate, nicotine sulphate, and summer oil on apples and also controlled cedar-apple rust better than sulphur although not so well as Fermate. Four years' consecutive trials in Pennsylvania showed 2-heptadecyl glyoxalidine (341) to be superior to other fungicides tested, including 2-8-100 Bordeaux, in controlling the defoliation of sour cherries (Montmorency) caused by leaf-spot. Although this resulted in somewhat duller color, there was no reduction in size of fruit such as that brought about by Bordeaux.

REPORT OF THE THIRD ANNUAL MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The third annual meeting of the Potomac Division of The American Phytopathological Society was held February 19 and 20, 1946, at the Plant Industry Station, Beltsville, Maryland. Officers elected for 1946-47 were E. E. Clayton, President; V. F. Tapke, Vice-President; W. F. Jeffers, Secretary-Treasurer; and R. J. Haskell, Councilor.

ABSTRACTS OF PAPERS PRESENTED AT THE THIRD ANNUAL MEETING OF THE POTOMAC DIVISION

Soil Fumigation against the Golden Nematode. CHITWOOD, B. G. The product D-D appears to be the most economical and effective soil fumigant used against the golden nematode of potatoes. Hand application of D-D at 425, 850, and 1700 lbs. an acre gave fumigation efficacy of 0.999, 0.996, and 0.999, respectively, with odds of 99:1 that treatments were better than 99, 98, and 99 per cent effective. Experimental plots were surrounded by clean fields. Hand application of D-D at 425, 675, 850, and 1275 lbs. an acre gave efficacy of 0.974, 0.982, 0.985, and 0.989, respectively, with 99:1 odds that treatments were better than 94, 95, 96, and 96 per cent effective. Plots were surrounded by infested fields. Machine-applied D-D at varied dosages per acre on 2 half-acre subplots per treatment gave the following efficacies: 452 lbs.—0.998 and 0.994 (better than 97 per cent); 631 lbs.—0.974 and 0.983 (better than 93 per cent); 858 lbs.—0.928 and 0.986 (better than 83 per cent); 930 lbs.—0.987 and 0.716 (second plot a failure); 1213 lbs.—0.991 and 0.973 (better than 93 per cent). Total overall efficacy was 0.938, with 99:1 odds that treatments were better than 86 per cent effective. There were 15,255 females observed in controls, 953 in treatments; of the latter, 693 were in one subplot. Mechanical failure might explain the single exceptional subplot.

Aerosol Treatments for the Control of Tobacco Blue-Mold Disease. CLAYTON, E. E. The benzyl salicylate-cottonseed oil blue-mold spray has been adapted to the aerosol type of treatment and used successfully 2 years. The concentrate used was benzyl salicylate 4 ounces and cottonseed oil 1 gallon. By one method cartridges were loaded with 40 per cent concentrate and 60 per cent Freon. By another method small high pressure sprayers were loaded with a mixture of 43 per cent concentrate and 57 per cent acetone. Air pressure in the sprayer was adjusted to about 110 pounds. The rate of application was controlled by regulation of the spraying time. Small plants received 6 minutes spraying per 100 square yards and large plants up to 14 minutes. Excellent blue-mold control was secured.

Tobacco Resistant to Root Knot and Nematode Root Rot. CLAYTON, E. E., and T. W. GRAHAM. Root knot (*Heterodera marioni*) and nematode root rot (*Pratylenchus* sp.) are common and serious tobacco diseases through the flue-cured region of the South-east. Search for resistance yielded one collection, T.I. 706, from which genotypes were selected that were highly resistant to both root knot and nematode root rot. This resistance has been consistent under the most severe field conditions in widely scattered locations. The full resistance was recovered after a cross with susceptible tobacco and after 1st and 2nd back crosses. Studies of back-cross lines showed segregation in all F_2 lines, but some F_1 lines were homozygous for resistance. Homozygous F_1 and F_2 lines have been secured for the 2nd back cross that appeared slightly more resistant to both root knot and nematode root rot than the original selection, T.I. 706.

Strawberry Virus in Eastern United States. DEMAREE, J. B. Preliminary studies indicate the presence of a virus disease of the yellows type in strawberries in Eastern United States. Distribution and prevalence have not been fully determined. There is some vein-clearing as well as a chlorosis of a transitory character in some varieties, appearing (if at all) in spring and fall, but the most pronounced and constant symptom is dwarfing due to short and horizontal growth of the petioles. Some varieties appear to be symptomless carriers. Artificial transmission by the stolon-grafting method in the greenhouse has been used during the past 3 years with a few eastern named and nameless varieties. The variety Marshall has proved satisfactory as an indexing plant.

Rate of Spread of Blueberry Stunt in a North Carolina Field. DEMAREE, J. B. The rate of spread of stunt (a virus disease) of blueberries varies greatly in different sections

and in fields in the same section. Its natural rate of spread is moderate to rapid in New Jersey, very rapid in some fields in North Carolina, and low to nil in Massachusetts, Michigan, and New York. Rate of dissemination in fields is undoubtedly associated with the presence and abundance of a vector. A part of a rectangular blueberry field (consisting of 10,000 plants set in 1936) was surveyed for 4 consecutive years for rate of spread of the disease. In lieu of surveying the entire field, 3 widely separated 10-row blocks of 600 bushes each were selected for surveying and designated as blocks 1, 2, and 3. Block 1 was laid out across the field near one end, block 2 was a section across the middle of the field, and block 3 was across the opposite end. Rate of spread of the disease in the 3 blocks from 1942 to 1945, inclusive, as indicated by percentages of affected plants, was as follows: Block 1—1942, 9.0; 1943, 23.3; 1944, 31.7; and 1945, 47. Block 2—1942, 4.7; 1943, 13.7; 1944, 23.7; and 1945, 47. Block 3—1942, 0.7; 1943, 1.5; 1944, 3.3; and 1945, 7.8. A few infected bushes were known as early as 1940 in block 1. The disease spread rapidly in that end of the field, and now nearly 50 per cent of the plants over at least half of the field are affected. After 6 years it has also become well established at the opposite end, a distance of about 200 yards.

Occurrence of a Strain of Tobacco-Etch Virus on Sweet Peppers in the Field. DOOLITTLE, S. P. Sweet peppers in Maryland and New Jersey occasionally have a virus infection which mottles the young leaves with small ring markings. Older leaves may have large, necrotic rings and eventually drop. Fruits of such plants often have large, concentric ring patterns and drop before maturity. These symptoms are largely confined to strains of the variety California Wonder and seldom appear on World Beater. A strain of tobacco-etch virus has been present in such plants. Inoculations with this virus alone, or in combination with the ordinary tobacco-mosaic virus or the cucumber-mosaic virus, have not produced ring markings of the fruit or leaves, although etch virus alone has produced leaf mottling similar to that seen in the field. When peppers are inoculated with both the etch and the tobacco-mosaic viruses, however, the leaf-mottling resembles that of etch virus alone, but there is severe defoliation of the plants. Fruits yellow and shrivel and have small, raised areas that produce a pebbled appearance. Fruit symptoms appear on California Wonder but rarely on World Beater. A disease of this type has caused serious losses in the field. With the combined infection, the defoliation has been more rapid than that sometimes caused by tobacco mosaic alone and, while the latter virus occasionally causes some yellowing and shriveling of the fruit, the fruit symptoms caused by the combined infection have been more consistently severe and of a typical character.

*High Resistance to Common Tobacco Mosaic in Certain Lines of *Lycopersicon hirsutum*.* DOOLITTLE, S. P., W. S. PORTE, and F. S. BEECHER. The wild tomato species, *Lycopersicon hirsutum* Humb. and Bonpl., is very tolerant of both yellow and green strains of the common tobacco mosaic virus. The majority of plants inoculated have no symptoms, but virus is present in such plants in varying and fairly high concentrations. In 1941, however, two plants without symptoms when inoculated with a yellow strain of the virus, were found to be virus-free when tested by inoculations on *Nicotiana glutinosa*. Further inoculations produced no infection and cuttings from the plants have been carried as clonal lines. Continued inoculations, chiefly with yellow strains of the virus, have produced no infection in any of the 38 cuttings of one line tested during the past 4 years. All plants were young and growing vigorously and often were inoculated more than once. In the second line, 5 of 42 cuttings have had a trace of virus after inoculation but inoculations of the remaining plants failed to cause infection. Owing to difficulty in securing seed from *L. hirsutum*, trials with seedling progenies of these lines have been limited, but 21 of 58 seedlings tested have had no infection. Crosses between these lines and varieties of the cultivated tomato have yielded tolerant individuals, but none have had the high resistance of the wild parents.

Factorial Studies on Dithane Plus Zinc Sulphate-Lime: the "Reaction Product" (Zinc Ethylene Bisdithiocarbamate). HEUBERGER, J. W. In 1943 the addition of zinc sulphate and lime to Dithane (disodium ethylene bisdithiocarbamate) increased the control of potato early blight from 5 to 90 per cent and increased yield from 154 to 220 bu. an acre. Factorial studies in 1944 on potato early blight showed that the addition of lime alone to Dithane did not increase control, that the addition of zinc sulphate alone markedly increased control, and that the addition of both zinc sulphate and lime was slightly less effective than adding zinc sulphate alone. This result suggested the preparation of the reaction product of Dithane-zinc sulphate, a white flocculate, as a dry powder. Such a preparation was made, at the writer's request, by the Rohm and Haas Company in 1945. The flocculate was filtered, dried, and brushed through a fine-mesh screen. Control of potato early blight in 1945 was: Check, 0 per cent; Dithane, 15; Dithane plus zinc sulphate, 87; Dithane plus lime, 15; Dithane plus zinc sulphate-lime,

80; Reaction Product powder, 87. Yields were, respectively: 76, 93, 156, 101, 157, and 178 bu. an acre; yield with Bordeaux was 135 bu. an acre. No injury was observed from the Reaction Product powder, which, chemically, is zinc ethylene bisdithiocarbamate.

Apparatus and Small Scale Field Plot Design for Evaluating Fungicides on Vegetables. HEUBERGER, J. W., and T. F. MANNS. The pathologist who deals with field screening of numerous potential fungicides is faced with two problems: (1) the most efficient apparatus and (2) the most economical plot design. Where 50 or more compounds are evaluated in replicated, randomized block designs it is obvious that commercial sprayers or dusters require too large a plot size; knapsack sprayers and dusters require too high a labor output. Apparatus and plot design used in Delaware is as follows: A $3\frac{1}{2}$ -gallon-per-minute pump, operated by a small air-cooled motor, is mounted on skids. This is transported in a two-wheel trailer of sufficient size to carry 500 ft. spray hose, 500 ft. garden hose, five 15-gallon barrels, spray booms, etc. Sprays are mixed in 15-gallon barrels; as one is sprayed out the other is prepared. Spraying is done with a hand boom having 2 nozzles on top and two at the sides. Plots are laid out in a long rectangle having one block on each side of a center driveway, and each block is divided, thus making 4 replicates. Replicate size is 20 plants for potatoes and 13 plants for tomatoes. The apparatus and plot design permit application per hour of 12 to 16 treatments on potatoes and 8 to 10 on tomatoes.

Investigations on the Possible Growth-Regulating Effect of Several Fungicides. JEFFERS, WALTER F. In the course of testing fungicides as dips for seed sweet potatoes it was observed that several materials caused a significant increase, over nontreated roots, in the number of sprouts produced, even when disease control was apparently not a factor. To test the possibility that some fungicides have a stimulatory effect on plant growth, several experiments on the application of such materials to bean plants were conducted. Spergon (tetrachloro parabenzoquinone) was the only material, which when applied either in lanolin or Carbowax 1500 to the stems of beans, consistently gave positive curvatures. This curvature was very slight as compared with that produced by indole acetic acid and 2-4-dichloro phenoxyacetic acid. Several fungicidal materials were toxic when applied in the above manner; 1 milligram being the amount applied. As bean plants were not entirely satisfactory for this study, pieces of sweet potato were dipped in Spergon and in water and incubated for 9 days at room temperature. Spergon stimulated root growth and cell proliferation.

Regional Differences in Resistance of Hevea Selections to South American Leaf Blight. LANGFORD, M. H. Hevea rubber seedlings and clones assembled from many parts of the world have been exposed to South American leaf blight (*Dothidea ulrei*) in widely scattered tropical American nurseries during the past five years. Sensitivity of the fungus to weather conditions, together with regional variation in disease severity, emphasized the need of thorough resistance tests in more than one locality for reliable selection work. Groups of clones from several widely separated areas of the native habitat of *Hevea* have been grown under comparable exposure to blight in regional test plots in Costa Rica, Panama, Trinidad, Brazil, and Peru. Likewise, large populations of Hevea seedlings from jungle trees have been tested at the place of their origin and in other areas. Both clones and seedlings that have proved highly susceptible in some areas have been damaged slightly or not at all in certain other areas. Prolonged exposure, however, has brought many cases of increased disease severity as variants of the fungus appeared. Some clones have proved highly susceptible to practically all regional populations of the fungus, others have been susceptible to certain variants only, and still others—now recommended for commercial use—have been highly resistant in all areas.

Golden Nematode on Commercial Potatoes. MACHMER, J. H. Examination of graded tubers and accompanying soil from heavily infested fields has shown that such potatoes as prepared for shipping may contain up to 379,500 golden nematode cysts per carload. Attempts to eliminate cysts in adherent soil and attached to tubers, by a combination of dry brushing and washing, succeeded in removing 86 per cent of the cysts. Jet-washing tested in a preliminary way removed 90 per cent of the cysts. A higher percentage reduction in cysts by jet washing might be obtained with improved technique.

What Is Net Necrosis of Potato? MANNS, T. F. Severe net necrosis has been observed many times in the Central and Eastern States by the writer during the past 35 years during seasons of much leafhopper injury. Pathologists in northern potato-seed-growing states associate net necrosis with leaf roll, though they state these symptoms are not in the final stages of leaf roll. Appel and Orton, W. A., stated that net necrosis was not a reliable symptom of leaf roll. Entomologists, as far as I know, have

never associated net necrosis with leaf-hopper injury. Recently Severin has shown that a hopper may produce necrosis in beet with no virus present. In 1945 in a compatibility test of DDT with other sprays, two varieties of potato, Cobbler and Sebago, were northern grown and certified; two varieties, Green Mountain and Pontiac, were Delaware grown and 50 per cent of tubers had severe net necrosis. An experiment included four sprays with (a) DDT, (b) DDT plus calcium arsenate, and (c) unsprayed. The yield from the DDT plot was double that from the unsprayed plot. No leaf roll developed in the potatoes affected with net necrosis. Fortunately no fungus disease complicated the picture. Leafhoppers reduced the yield 50 per cent and net necrosis developed rapidly on the unsprayed potatoes in storage. The evidence is that net necrosis is leafhopper injury.

The Effect of Fungicides and Growth Substances on Easter Lily Bulb Production in the Field. McCLELLAN, W. D., and NEIL W. STUART. In August, 1944, 315 Easter lily bulbs (U.S.D.A. Clone 6, which is relatively resistant to *Fusarium* basal rot), averaging 8.6 inches in circumference, were scaled and the scales treated with tale, Arasan (tetramethyl thiuram disulfide), or Fermate (ferrie dimethyl dithiocarbamate), alone or containing 0.1 or 0.02 per cent of naphthaleneacetic acid (NA). The scales were planted in a 3 by 3 factorial in 5 bulb units in 7 randomized blocks in the field at Beltsville, Maryland. The bulbs were from 3 sources: (1) forced in Haydite in subirrigated beds; (2) forced in soil in pots; and (3) grown continuously in the field. In August, 1945, the bulbs were harvested, graded, counted, and weighed. There were no significant differences in the amount of basal rot between treatments. However, the greatest amount of basal rot occurred in those bulbs having the Haydite history (1), less in those with the greenhouse pot history (2), and none in those with the field history (3). Numbers of bulbs produced per 100 scales were as follows: tale, 393; 1: 1000 NA-tale, 309; 1: 5000 NA-tale, 379; Arasan, 513; 1: 1000 NA-Arasan, 538; 1: 5000 NA-Arasan, 418; Fermate, 488; 1: 1000 NA-Fermate, 515; 1: 5000 NA-Fermate, 456. Differences between treatments were greatest with the Haydite-history bulbs (1) and least with the pot-history bulbs (2). In 1943 and in 1944 Easter lily bulbs were treated with fungicides and combinations of fungicides with growth substances (16 different treatments in 1943 and 17 in 1944), but when the bulbs were harvested no one treatment could be rated as superior to the untreated controls.

The Effect of Fungicide-Growth-Substance Combinations on Herbaceous Cuttings. McCLELLAN, W. D., and NEIL W. STUART. In an attempt to reduce losses due to rot, with fungicides, cuttings of geranium, snapdragon, chrysanthemum, and carnation were set in sand after dipping in dry tale, Spergon (tetrachloro parabenzquinone), Arasan (tetramethyl thiuram disulfide), and Phygon (2,3-dichloro-1,4-naphthoquinone) alone or containing 0.1, 0.04 or 0.02 per cent of naphthaleneacetic acid, naphthalene acetamide, or indolebutyric acid. The three fungicides reduced the percentage of rooting in all species except geranium. Undiluted Arasan severely injured chrysanthemum but not carnation cuttings. Spergon, however, was not injurious to chrysanthemum but severely injured carnation cuttings. Inclusion of 0.1 per cent of the growth substances in the fungicides increased percentage of rooting in tetraploid snapdragons and in one variety of chrysanthemum cuttings. In general naphthaleneacetic acid was the most effective of the growth substances. In other tests, dilution of Arasan and Fermate with tale (dilutions containing 1 to 20 per cent fungicide) prevented reduction in percentage of rooting of chrysanthemum and geranium cuttings. Addition of growth substances to the tale-fungicide mixtures increased the percentage and amount of rooting. Undiluted Fermate, Spergon, and Arasan used on poinsettia cuttings produced rooting and vigor ratings of 177, 139, and 107, respectively, as compared with 156 for tale. There was an insufficient amount of cutting rot in any of the tests to permit any conclusions as to the fungicidal value of the materials.

Golden Nematode as a Quarantine Problem. McCUBBIN, W. A. In 1944 a survey of potato fields in 19 northeastern States indicated that this potato root parasite is probably not long or widely established elsewhere than in Long Island. In 1945 intensive survey in all Long Island potato areas disclosed no infestation except in 5 additional farms in the infested area environs. A quarantine established by the State of New York in March, 1944, and revised February 15, 1946, controls outward movement of nursery stock, rooted plants, and topsoil, requires the cleaning of transported tools and implements, and restricts potato shipment to New York City or to chip and starch plants. A plan now under discussion proposes to prohibit potato and root crop production on infested land and give it a D-D treatment to minimize spread. Prospects for successfully dealing with this pest in the years to come are uncertain. The nemia may have been introduced from abroad into other areas as yet undiscovered; early potato shipments from infested Long Island farms may already have carried this pest elsewhere; and, finally, no known method

of survey can detect small, incipient outbreaks except by chance, so that new centers may remain undiscovered until well developed.

Progress on Tropical American Rubber Planting Through Disease Control. RANDS, R. D. Since 1940 the U. S. Department of Agriculture has conducted cooperative research and furnished technical guidance to 13 tropical American Republics interested in rubber planting. Control of leaf blight (*Dothidella ulei*) by spraying, as developed by Langford, and by crown budding with resistant Ford selections has removed the main obstacle which in the past nullified all attempts to establish tropical American plantations. These effective and economical procedures have enabled immediate use of the highest yielding Oriental clones, all of which are very susceptible to leaf blight. Commercial planting has now emerged from the nursery stage in several of the countries, and to the end of 1944, more than 28,000 acres of high-yielding rubber had been established. Development of a permanent, self-sustaining, small-farm or single-family type of rubber production is emphasized. This has required adjustment of local farm credit, colonization and other programs of the cooperating governments, which together with their nationals have reported total expenditures through 1945 of about \$3,844,000 on their rubber projects. While the planting of crown-budded Oriental clones has proceeded, a cooperative breeding program has furnished thousands of first and second generation hybrids, some of which have given indication of combining superior yield with blight resistance.

Effect of Seed Treatment on Soybeans Germinated at Four Temperatures. SHERWIN, HELEN S., C. L. LEFEBVRE, and R. W. LEUKEL. Seedling emergence of soybeans was significantly increased when the seed was treated with Arasan (tetramethyl thiuram disulfide), New Improved Ceresan (ethyl mercury phosphate), and Spergon (tetrachloro para benzoquinone), and planted in soil maintained at 15°, 20°, and 25° C. At 30° C., however, there was no significant increase in emergence. The soil in all cases was adjusted to 55 per cent water holding capacity. In the 12 seed lots tested, greater improvement in emergence was obtained from the treatment of seed that was produced in Mississippi than from similar treatment of seed produced in Illinois. Seed lots having low germination seemed to be benefited more by Arasan and New Improved Ceresan than by Spergon.

The Present Status of Breeding for Disease Resistance in Oats. STANTON, T. R. The most important new disease-resistant varieties have been developed by crossing Richland on Victoria, resulting in Vieland, Tama, and similar varieties, grown on about 25,000,000 acres in 1945. Less important groups of disease-resistant winter oats have been developed by crossing Fulghum, Nortex, and Lee on Victoria and by crossing Fulton type early red spring oats and Victoria-Richland selections. New varieties developed from crosses on Bond with better resistance to crown and stem rusts, greater productiveness, higher test weight, and stiffer straw are being released. These include Clinton and Benton in Iowa, Indiana, and Illinois; Bondu and Mundo in Minnesota; and Eaton in Michigan. Forvie [Forward × (Victoria-Richland)] is a promising new disease-resistant variety in Wisconsin. Threatening new or uncommon races of smuts, rusts, and other diseases are becoming important, such as: Races of smut that are virulent on Victoria, Boone, Vieland, Lectoria; race 45 and similar races of crown rust that infect Bond, Clinton, and Benton; races 8 and 10 of stem rust; *Helminthosporium* diseases on recently distributed varieties; and oat viruses in the Southeastern States. Evidently, much yet remains to be done if all these diseases are to be more completely controlled by breeding. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture and State Agricultural Experiment Stations.)

Distribution, Host Range, Character, and Significance of the Golden Nematode as a Disease Agent. STEINER, G. The golden nematode, *Heterodera rostochiensis* Wollenweber, occurs in Great Britain (including Ireland and Jersey Island), Sweden, Denmark, some northern and northwestern Provinces of Germany, and Long Island, U.S.A. It was known as potato dab or potato sick soil in Yorkshire around 1903; Zimmermann in Mecklenburg first recognized a nematode as its cause (1913) and Wollenweber described it as a separate species (1923). It was first observed in Sweden in 1922; in the United States in 1941. White potato and tomato are the main hosts; certain solanaceous weeds, also *Atropa belladonna* L. and *Chenopodium album* L., have been reported attacked. Tests by Ellenby showed some 50 tuber-forming South American species of *Solanum* invaded but generally to a lesser degree than white potato. *H. rostochiensis* lives sedentary on roots and tubers, the female transforms into a cyst remaining in the soil up to 10 years and containing viable eggs up to 8 years. This longevity of the cysts and the fact that tubers of affected plants appear healthy make it a particularly dangerous pest, usually not recognized before large infestations have been built up. Crops have been reduced to less than seed. An attempt to eradicate it on Long Island is planned.

Seed Treatment of Castor Beans for the Control of Seedling Blight. STEVENSON, E. C. Seedling diseases of castor beans caused pre-emergence and post-emergence blight or die-back; however, many affected plants which were not killed developed normally after the first true leaves were formed. In such plots the plants were later in maturing and the yield was reduced, but the quality of the seed was not affected. Seed treatment tests were made in 1943, 1944, and 1945. In 1943 the use of Semesan (hydroxymercurichlorophenol), New Improved Semesan Jr. (ethyl mercury phosphate), Spergon (tetrachloro parabenzoquinone), Arasan (tetramethyl thiuramdisulfide), and Thiosan (tetramethyl thiuramdisulfide) resulted in significantly better emergence. In 1944 and 1945 Semesan and New Improved Semesan Jr. were dropped from the test because of the shortage of mercurial compounds at that time. Arasan, Fermate (ferric dimethyl dithiocarbamate), and Spergon were tested at Manhattan, Kansas, and Stillwater, Oklahoma, in 1944, and at Beltsville, Maryland, in 1944 and 1945. The use of Arasan improved emergence significantly at Manhattan, Kansas, but none of the treatments was effective at Stillwater, Oklahoma. (Very little disease was present in the Kansas-Oklahoma areas during the year of the test.) Emergence was improved significantly by all three treatments in the two-year test at Beltsville, Maryland. Arasan was more effective than either Spergon or Fermate, the latter two not differing from one another statistically. Plant vigor and seed yield were not affected by any of the treatments.

A Simple, Effective Method for Inoculating Barley Seed with Helminthosporium gramineum. TAPKE, V. F. It has been difficult to develop a satisfactory simple method for the artificial inoculation of barley with the stripe fungus, *H. gramineum*. The following new method is relatively easy to apply and has consistently resulted in high infections with only small reductions in seed germination: The stripe fungus is grown on a nutrient broth in half-liter Erlenmeyer flasks for 10 days at room temperature, the mycelial pads are then fragmented in a homogenizer and applied in aqueous suspension for 15 minutes to seed that has been thoroughly dried following a 6-hour soak in water at approximately 25° C. During the first minute of the soak, the vessel containing the seed and inoculum is vigorously shaken. Next the inoculated seed is drained and incubated on moist blotters at 7° to 10° C. for 4 days, then dried at room temperature and sown. Through this method up to 100 per cent striped plants have been obtained in susceptible winter barleys grown in the field.

Spread of the Dutch Elm Disease in Maryland. WALKER, E. A. The Dutch Elm disease (*Ceratostomella ulmi*) became a serious menace to elm trees in Frederick County, Maryland, during 1944-45. The first diseased tree in Maryland was found at Ft. McHenry, Baltimore, in 1933. An outbreak was checked at Brunswick, Frederick County, in 1935, and near Cumberland, Allegany County, in 1936. No outbreaks have occurred in Baltimore or Cumberland areas since 1941. A light outbreak was observed in 1943, on 8 trees in Frederick County. This increased to 29 diseased trees in 1944, and five cases were reported for Carroll County. The elm bark beetle (*Scolytus multistriatus*) population increased greatly in 1944, and by 1946, 261 confirmed trees were found in Frederick County; 5 in Carroll, and 1 in Washington County. The disease is now confined mostly to the Monocacy River Valley, and its feeder streams. During 1944-45 State Officials removed and destroyed 101 diseased elm trees in Frederick County, 7 in Carroll County, and one in Washington County; and 245 other bark-beetle infested, dead and dying elms in Frederick County; and 47 in Carroll County. Since 1933, 120 confirmed elm trees have been removed in Maryland. The wet 1945 season greatly reduced the elm bark beetle population in Maryland.

Field Control of Bean Rust with Sulfur. ZIMMEYER, W. J. During the 1945 season bean rust in Pinto and Great Northern bean acreages was well controlled in Colorado, Wyoming, and Montana with sulfur dust applied at the rate of 20 to 25 pounds per acre. In the bean-growing sections of these States environmental conditions were ideal for the spread and development of rust. Early in the season infection is usually sparse and the control at this time is relatively simple, thus preventing secondary spread. In the Greeley, Colorado, area one dusting applied by most growers in early July, before the plants covered the rows, practically eliminated the disease. In Wyoming only a small percentage of the fields were dusted. Fields dusted twice yielded on an average of 1600-1800 pounds of seed per acre while fields not dusted averaged from 800 to 1000 pounds. In the Bridger, Montana, section where rust was widely distributed, 9 fields (totaling 330 acres) dusted twice averaged 2000 pounds of clean seed per acre. Eight fields (comprising 164 acres) dusted once averaged 1400 pounds, and 18 fields not dusted (totaling 364 acres) averaged 1016 pounds per acre. The best-producing, twice-dusted field yielded 2369 pounds and the poorest of the non-dusted fields yielded 380 pounds of seed per acre.

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THE FIRST SYMPTOM OF TOMATO FUSARIUM WILT: CLEARING OF THE ULTIMATE VEINLETS IN THE LEAF

R. E. FOSTER¹

(Accepted for publication March 13, 1946)

The later symptoms of typical *Fusarium* wilt of the tomato, caused by *Fusarium oxysporum* f. *lycopersici* (Sacc.) S. & H., have been described by many workers since the disease was first seen prior to 1895 (1, 4). The symptoms most commonly mentioned have been stunting, progressive yellowing and wilting of the leaves, wilting and collapse of the stem, and death of the plant, all accompanied by an increased darkening of the vascular elements. Wellman (5) recently described epinasty as an early symptom of tomato wilt. A still earlier symptom of this disease has been seen repeatedly, and it is believed to be the first above-ground indication of infection of the tomato by the wilt pathogen.

This early symptom is a clearing of the ultimate veinlets in the leaflets of infected tomatoes giving them a "netted" appearance (Fig. 1). It can be seen only when leaves are viewed with transmitted light. In a detailed study of 50 young tomato plants (4 to 6 leaves) inoculated with a virulent strain of the organism and held at optimum conditions for wilt development (optimum moisture, 28° C.) and observed at 12-hour intervals, it was noticed that veinlet clearing appeared on many plants 24 hours after dip-inoculation. Most often it was first evident in the terminal leaflet of the third leaf, after 36 hours appearing in the second and fourth leaves and later in all of the leaves. Veinlets closest to the main veins became cleared first and the large veins themselves seemed to be outlined by a very narrow, cleared band. Occasionally, this clearing progressed more rapidly on one side of the leaflet. The loss of green color soon progressed along all veinlets and later extended into the "vein-islets" which, just previous to veinlet clearing, appeared to be a darker green. As the clearing spread from the veinlets, yellowed areas were formed in the leaf which later coalesced, giving rise to the "yellow-leaf" symptom of the disease. Forty-eight hours after dip-inoculation veinlet clearing was present in all leaves. Unilateral inoculation of tomato plants with the fungus occasioned a unilateral development of this early symptom. Epinasty did not appear in any of the plants until 72 hours after inoculation, and in most it was not evident until after 84 hours. Veinlet clearing seems to be associated entirely with the effects of *Fusarium oxysporum* f. *lycopersici* on the tomato host. Noninoculated plants held under the same conditions did not have the veinlet clearing symptom. Repeated attempts to recover the organism from affected areas in the leaf 36 hours after inoculation were unsuccessful. Likewise, attempts

¹ The writer wishes to express his gratitude to Eugene Herring for the preparation of the illustration. Investigation supported in part by a grant from the Wisconsin Alumni Research Foundation.

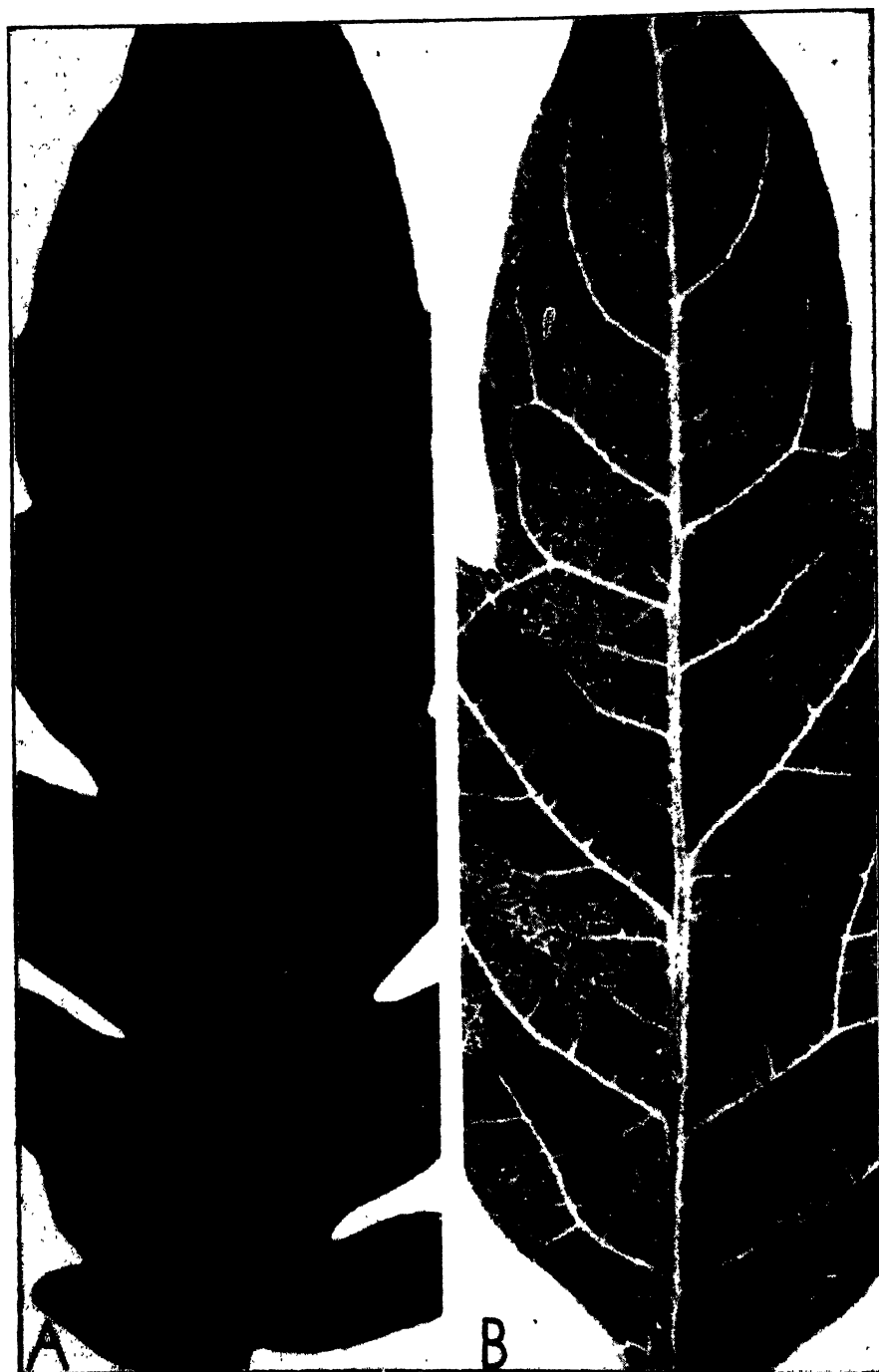


FIG. 1. Portions of terminal leaflets from (A) a healthy tomato plant, and (B) an inoculated tomato plant: $\times 4$.

to produce the symptom on healthy tomato and tobacco leaves by rubbing them with juice expressed from affected leaflets met with no success. Symptoms identical to those described have not been seen in association with any other tomato diseases, although it is recognized that other vascular parasites might produce toxic materials which could bring about a similar effect.

Clearing of the ultimate leaf veinlets of inoculated tomato plants has been observed in several host varieties and under various environmental conditions. Bonny Best, Marglobe, Master Marglobe, and Rutgers (*Lycopersicon esculentum* Mill.) varieties all showed the early symptom. However, the time interval between inoculation and first appearance of veinlet clearing varied with the relative resistance of the variety. It has not been found in a highly resistant strain of Red Currant tomato (*L. pimpinellifolium* Mill.) or in highly resistant hybrids. In inoculated susceptible plants grown under a wide range of environmental conditions (3), the production of the veinlet-clearing symptom was not affected by soil moisture, soil temperature, air temperature, light conditions, or host nutrition, except in relation to time of appearance, as long as the particular environment permitted an ultimate development of tomato Fusarium wilt. The three strains of *F. oxysporum* f. *lycopersici* tested brought about development of the early symptom. These three strains were (a) virulent, raised strain R 5-6, (b) mild, appressed strain A 15-8, both obtained from F. L. Wellman (6), and (c) a strain of intermediate pathogenicity from J. B. Kendrick, California. The rapidity with which the netting symptom became evident varied with the pathogenicity of the fungus strain.

Numerous attempts were made to produce the early, veinlet-clearing symptom in young plants placed in fungus extracts in a manner similar to that used by Fisher (2). Young Bonny Best plants (5 to 6 leaves) were grown in soil, were dug, the roots were cut off under water, and then the stems were placed in filter-sterilized Richard's nutrient in which the virulent strains of the *Fusarium* had been growing for different lengths of time. Veinlet-clearing symptoms appeared only in plants placed in the medium from an agitated 28-hour culture. Media from older cultures produced yellowing and necrosis rapidly, without signs of veinlet clearing; younger cultures were not used. Water and Richard's solution controls produced no symptoms.

Very early symptoms of tomato Fusarium wilt are of little importance in studying the disease as it occurs in the field, but such symptoms have been of considerable value in various controlled studies in the greenhouse and laboratory. Moreover, the rapid appearance of evidence of host-parasite interaction suggests a phase of disease development that has received little consideration. It is widely accepted that the disease effects brought about by vascular fusarial infection are occasioned largely by toxin produced in the infected root and spread throughout the plant in the transpiration stream. The appearance of a disease-induced effect in the upper portion of a plant only 24 hours after root inoculation indicates that toxin

is produced at the time of fungus penetration or very shortly thereafter, and certainly before the organism is well established within the host tissue. Whether the toxin causing veinlet clearing is produced by the fungus or by the plant in response to fungus action has not been shown definitely. However, the former possibility seems the more likely in view of the fact that the same peculiar symptom can be induced by aseptic fungus extract. The observations presented seem to contribute to the theory that the disease reactions brought about by vascular *Fusaria* are conditioned by the formation of more than one type of toxin depending upon the degree of parasite invasion and consequent host reaction. †

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BACTERIAL LEAF SPOT AND BUD ROT OF ORCHIDS CAUSED BY PHYTOMONAS CATTLEYAE

PETER A. ARK AND H. EARL THOMAS

(Accepted for publication April 26, 1946)

A large number of orchid-growing establishments in California often sustain heavy losses from brown-spot disease in such valuable species as *Cattleya* and *Phalaenopsis*. Microscopic examination of diseased specimens has always revealed the abundance of bacteria in advancing margins of the spots. In order to ascertain the causal agent of the disease a bacteriological study of the isolates was made. This paper presents the results of the investigation as to the nature of the disease, the causal organism, and the method of control.

Elliott (1) lists the following organisms as causal agents of disease in orchids: *Bacillus cypripedii*, *Bacillus farinetianus*, *Bacillus pollacii*, *Bacterium cattleyae*, *Bacterium krameriani*, and *Bacterium oncidii*. These organisms attack orchid leaves and pseudo-bulbs, causing occasional death of the plants. Recently (3) a soft rot of *Cattleya sp.*, caused by *Erwinia carotovora*, was reported in the United States.

Erwinia carotovora was also reported as a pathogen on *Phalaenopsis aphrodite* in Japan (4) and *Cymbidium aloifolium* and *C. insigne* were successfully inoculated. The disease starts as water-soaked spots on the leaf blades, which become sunken and brown. The organisms enter the plant through wounds. Matsumoto and Okabe (4) presented evidence which indicates that the *Bacillus cypripedii* of Hori (2) is very closely related to, if not identical with, *E. carotovora*.

For a number of years the writers have studied a disease of orchids which occurs in *Phalaenopsis sp.* and *Cattleya sp.* and sometimes causes considerable damage in orchid houses in the San Francisco Bay region. The disease is prevalent in greenhouses in which a high air humidity is maintained for considerable time and where the plants are syringed. A bacterial organism was isolated from the advancing margins of the lesions, proved pathogenic upon inoculation, and was readily reisolated.

At first the disease expresses itself as small, dark, water-soaked spots, which rapidly increase in size, changing from light-brown to dark chestnut brown with age. These spots upon enlarging may coalesce to form larger areas (Fig. 1, A and B). Under favorable conditions of temperature and moisture the infection spreads rapidly and may attack the crown, sometimes killing the plant. The disease may be initiated through wounds, but the organism can apparently cause infection by direct penetration of uninjured leaves.

Phalaenopsis sp. and *Cattleya sp.* were infected under conditions which approximated those obtaining in commercial greenhouses. The following orchids were susceptible when artificially inoculated: *Epidendrum o'brein-*

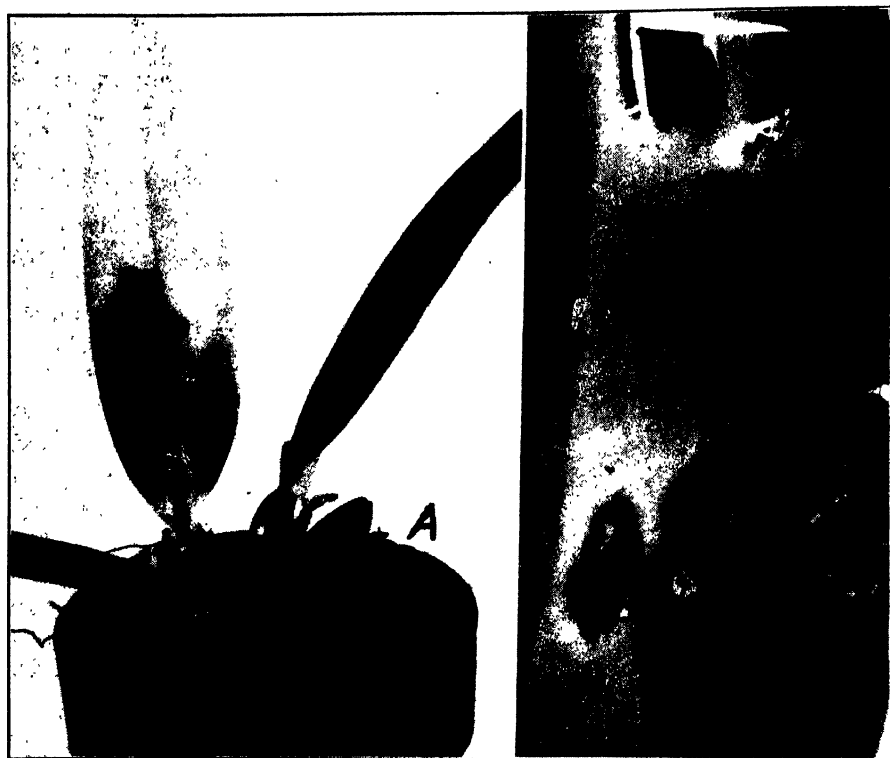


FIG. 1. Bacterial leaf spot on *Cattleya* sp. A. Natural infection. B. Detail of a well advanced sunken spot on ventral side of a leaf.

ianum, *Dendrobium* sp., *Cypripedium* sp., *Phalaenopsis amabilis* and *Vanilla*. No infection could be obtained on *Phalaenopsis lueddemanniana*.

Four isolates of the organism, together with four reisolates, were studied bacteriologically in order to ascertain the taxonomic position of the causal agent. All cultures behaved identically in the laboratory and had certain features ascribed to *Bacillus farctianus*, *Bacillus pollacii*, *Bacterium cattleyae*, and *Bacterium kramertianii*. However, as Elliott (1) pointed out, there is no certainty that the work on these four species was done with pure cultures. All were imperfectly described, so that with few exceptions they would be interchangeable (Table 1).

TABLE 1.—Comparison of cultures causing orchid leaf spots according to Pavarino (5)

<i>Bacterium cattleyae</i>	<i>Bacillus farctianus</i>	<i>Bacterium kramertianii</i>	<i>Bacillus pollacii</i>
2.4 μ \times 0.4–0.6 μ	1.5 μ \times 0.8–1.0 μ	2–3 μ \times 0.6–0.8 μ spore-forming	8–10 μ \times 1.0 μ
Gram-negative	Gram-positive	Gram-negative	Gram-negative
White on agar	Iridescent to grayish on agar	Greenish on agar	Greenish on agar
Gelatin not liquefied	Gelatin liquefied	Gelatin liquefied	Gelatin liquefied

A description of the organism causing the disease occurring in California is given below. This bacterium is probably the one which was described by Pavarino (5) as *Bacterium (Phytomonas) cattleyae*, since all the characters of this organism, as indicated by Pavarino, including the symptoms of the disease, are in close agreement.

Phytomonas cattleyae. Small, Gram-negative rods in stained smears of 24-hour-old beef-extract-peptone-agar growth, single and in pairs; motile by means of one or two lophotrichous flagellae; no spores as determined by staining and by heating to 60° C. for 20 minutes.

On beef-extract-peptone agar of pH 6.9, at 28° C., growth is rapid, grayish-white with iridescence when examined by transmitted light. The consistency is butyrous. The colonies are large, entire, smooth, with criss-cross markings resembling fish scales. On potato-dextrose-peptone agar slants the growth is very rapid and dirty-gray. On potato plugs the growth is rapid and slimy, dark-gray. Beef-extract-peptone broth becomes very turbid within 24 hours with the formation of a very delicate pellicle which later drops to the bottom, leaving a rim of growth on the walls of a test tube. Good growth occurred in liquid synthetic media such as: synthetic carbohydrate media of the Society of American Bacteriologists (6, p. 15), Fermi, Cohn, and Uschinsky. Starch is digested slowly. Gelatin is not liquefied even after thirty days. Hydrogen sulphide and indol are not produced. Nitrates are reduced to nitrites. Both skimmed and litmus milk were unchanged when the cultures were observed 3, 7, and 14 days after inoculation. Ammonia is produced in nutrient broth after 3 days, determined by the method of Hansen (7, p. 15). Acid and no gas is produced in a synthetic carbohydrate medium of the Society of American Bacteriologists (6, p. 15) to which one per cent of arabinose, dextrose, dulcitol, galactose, glycerol, lactose, levulose, mannite, sucrose, or xylose was added and as an indicator 0.5 cc. of a one per cent alcoholic solution of brom cresol purple and 0.4 cc. of a one per cent solution of cresol red to each liter of the medium and a Dunham fermentation vial (inverted) to each tube to detect gas. Neither acid nor gas was formed from raffinose. The optimum temperature for growth lies between 25° and 35° C. The thermal death point is 48° C.

To obtain satisfactory control of the disease, badly diseased plants should be removed and individual spots on the leaves treated by carefully swabbing with a sponge soaked in 1 to 1000 corrosive sublimate (HgCl₂). No injury of the treated leaves was observed, either on *Cattleya* or *Phalaenopsis*. A considerable reduction in the disease was obtained when the greenhouse humidity was decreased and overhead irrigation discontinued.

SUMMARY

1. A bacterial leaf spot and bud rot of *Cattleya* sp. and *Phalaenopsis* sp. caused considerable damage in orchid houses in Central California.

2. The disease may be controlled by reducing the air humidity and by swabbing diseased plant parts with 1 to 1000 corrosive sublimate solution.

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SEEDLING DISEASE OF YELLOW CALLA, CAUSED BY CORTICIUM SOLANI, AND ITS CONTROL

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INTRODUCTION

A seedling disease of the yellow calla (*Zantedeschia elliottiana* Engler) has long been prevalent in field-planted seedbeds in and near Santa Cruz, California. Annual losses ranged from 25 per cent or more, with occasional complete failures. The results of field and laboratory studies of this disease during the past 5 years, with suggestions for control, are presented in this paper.

SYMPTOMS OF THE DISEASE

Yellow calla seedlings may become infected before or after emergence. The symptoms of the disease on the roots of infected plants consist of a dry, brownish-black discoloration of the cortical tissues, generally commencing at the tip of the roots and progressing toward the crown. Sometimes infection occurs above the tips of the roots. The cortex of infected roots soon shrivels and sloughs off, exposing the central cylinder. Seedlings infected before emergence are small and die very quickly. This phase of the disease can only be observed by digging into the soil just before the seedlings emerge, which usually occurs in from 4 to 6 weeks after sowing the seed. The post-emergence phase of the disease is equally destructive. Apparently healthy seedlings with 2 to 6 leaves may suddenly turn yellow, wilt, and collapse. Death ensues in a day or two and may affect single seedlings or a group of them in the same row. When removing diseased seedlings from the soil, little or no pressure is required. A third and equally important phase of the disease is rotting of the seed before germination; this is also an important factor in the reduction of stand. Rotting of the seed and seedling infection before and after emergence can usually be observed in any seedbed. However, seasonal variations, coupled with unfavorable cultural practices, may accentuate the loss from seed decay or, on the other hand, seedling mortality (pre- or post-emergence) may be the most conspicuous feature of the disease.

This disease occurs on widely different soil types, ranging from sandy and gravelly loams to heavy clay, and is favored by excessive irrigation, poor drainage, warm weather, improperly prepared seed, mulching, and crowding of the plants.

THE CAUSAL FUNGUS

Diseased yellow calla seedlings, showing different stages of root infection, were collected at Capitola and Santa Cruz each summer from 1941 to 1945, inclusive, for cultural tests in the laboratory. After washing the roots in tap, distilled, and sterile distilled water, followed by drying on clean filter paper, small bits of root tissue were planted on potato-dextrose agar in

Petri dishes. Isolations each year consistently yielded but 1 fungus which has been identified as the vegetative stage of *Corticium solani* (Prill. and Del.) Bourd. and Galz. Isolates were obtained from hyphal tips of the fungus in Petri-dish cultures. Comparison of the isolates obtained each season from different seedbeds showed a remarkable similarity in macroscopic characters.

The relation of temperature to growth of the mycelium was determined for 2 isolates of *Corticium solani*, 1 from Capitola and 1 from Santa Cruz. The culture tubes (2.1 by 20 cm.) used and the procedure followed were those previously described by Tompkins and Gardner.¹ The medium used was potato-dextrose agar, pH 5.8. Inoculated tubes were kept at room temperature for 24 hours. Then 3 tubes of each isolate were placed in a horizontal position in controlled temperature chambers at intervals of 3°, from 4° to 40° C. The cultures were incubated for 96 hours. The cardinal temperatures were determined on the extent of mycelial growth in the culture tubes.

The minimum temperature for growth of the yellow-calla isolates of *Corticium solani* was approximately 7° C., the optimum 25° to 28°, and the maximum 37°.

These isolates were also used in the infection experiments in the greenhouse. Inoculum was prepared by growing the isolates on sterilized, moistened cracked wheat in 8-inch test tubes. When ready for use, the inoculum of either isolate was added to each of 6 flats of steam-sterilized, sandy-loam soil. After the soil and inoculum had been thoroughly mixed, the flats were set aside for 1 week. Simultaneously, controls were prepared by adding the same amount of sterilized cracked wheat to each of 4 flats of steam-sterilized soil. The soil in all flats was stirred and irrigated on alternate days before seeding. Each flat was planted at a depth of 1 inch with 500 untreated yellow calla seeds, obtained at Santa Cruz, at intervals of $\frac{1}{2}$ inch in the row, the rows being 1 inch apart. Prevailing greenhouse temperatures ranged from 14° to 18° C. After 5 weeks, seedlings appeared above ground in all flats. Disease was prevalent in all infested flats, as indicated by the yellow foliage, collapse of the seedlings, and skips in the row. In general, the symptoms on artificially-infected plants were identical with those of naturally-infected plants. Infection counts were made from the fifth to the ninth week, inclusive. The Capitola isolate caused a pre-emergence loss of 25 per cent and a post-emergence loss of 48 per cent, or a total of 73 per cent. The Santa Cruz isolate caused a pre-emergence loss of 32 per cent and a post-emergence loss of 51 per cent, or a total of 83 per cent. All yellow calla seedlings in the control flats remained healthy for the duration of the tests. The fungus was reisolated from 50 infected plants in each of the infested flats; the reisolates were identical with the original isolates. When reisolates of the Santa Cruz and Capitola isolates were tested, they proved highly pathogenic.

¹ Tompkins, C. M., and M. W. Gardner. Relation of temperature to infection of bean and cowpea seedlings by *Rhizoctonia bataticola*. *Hilgardia* 9: 219-230. 1935.

CONTROL OF THE DISEASE

Since field studies at Capitola and Santa Cruz suggested that certain cultural methods and lack of seed treatment were important factors related to the high incidence of infection in yellow calla seedbeds, control was predicated on improving the cultural conditions in the beds and on treatment of the seeds with a suitable fungicide.

Control experiments were conducted at Santa Cruz for 3 years. Briefly, the method of control was based on (1) thorough cleaning of the seed; (2) dusting the seed with an equal mixture of Spergon (tetrachloro parabenzoquinone) and Celite 505 (an infusorial earth used as a carrier); (3) wider spacing of seeds to prevent subsequent crowding of seedlings in the beds; (4) avoidance of excessive irrigation; (5) frequent cultivation of the soil to provide good aeration; (6) selection of a well-drained site; and (7) elimination of organic soil mulches.

Limited field tests, based on the foregoing procedure, were conducted in 1942, resulting in almost perfect stands of seedlings. In the control seedbeds, handled according to prevailing commercial practice, seedling disease was as destructive as in previous years. Because of the ease of applying Spergon and its effectiveness, no other fungicidal dusts were tested. In 1944 and 1945, the field tests were enlarged because more seed was available. Results were comparable with those obtained in 1942. The adoption of this method for controlling the disease in yellow-calla seedbeds has been recommended to the growers.

DISCUSSION

Improper processing of dried, yellow-calla spadices, in the fleshy pulp of which the seeds are imbedded, yields unclean seeds, *i.e.*, the seeds are partially or entirely covered with a layer of dried pulp. After these seeds are planted and the beds irrigated, the pulp attached to the seedcoats readily absorbs moisture and conceivably becomes a moist chamber surrounding each individual seed. Thus the way is prepared for infection by the pathogen. To circumvent this condition, it is suggested that the spadices be crushed and washed in tap water. Then the viable seeds, which sink to the bottom of the container, should be washed at least 4 times, dried on clean absorbent paper, and dusted with a fungicide, such as Spergon.

Close planting of yellow calla seeds, long an established commercial practice, insures crowding and predisposes the young seedlings to heavy infection. It would be desirable to plant the seeds 1 to 2 inches apart in short rows, the depth varying from 1½ to 2 inches, with rows 6 inches apart.

Daily irrigation of seedbeds is another common practice which favors the disease. Semi-weekly or weekly irrigation of the seedbeds would provide ample moisture for germination and growth and would aid in decreasing the amount of infection. The selection of a well-drained site is of paramount importance.

Some growers cover their seedbeds to a depth of 1 or more inches with

organic materials, such as macerated coffee husks, in order to conserve the moisture. Mulches not only interfere with good soil aeration, but conceivably they serve as moist chambers for fungus invasion. Their use should be avoided.

In late summer, in mature yellow calla plantings, the withering peduncles are unable to support the weight of the spadices, which lodge on the ground. Collections of spadices were made for 3 years in order to test the pulp and seeds in the laboratory for the presence of *Corticium solani*. The fungus was never isolated from these plant parts.

SUMMARY

A destructive seedling disease of yellow calla (*Zantedeschia elliottiana*) is prevalent in commercial plantings in the Capitola-Santa Cruz section of California. It is favored in its development and spread by warm weather, excessive moisture, poor soil drainage, the use of unclean, untreated seed, close planting of seed, and mulching.

The principal symptoms of the disease consist of a brownish-black discoloration of the cortical tissues of the fibrous roots (before and after emergence), usually commencing at the tips and progressing toward the crown. Invaded tissues shrivel and slough off. Above ground, infected seedlings develop yellow leaves which wilt, collapse, and die. Rotting of the seed may also occur before germination.

Corticium solani (Prill. and Del.) Bourd. and Galz. has been consistently isolated from infected seedlings and has proved pathogenic in greenhouse tests.

The minimum temperature for mycelial growth of the 2 isolates studied is 7° C., the optimum between 25° and 28°, and the maximum 37°.

The disease can be readily controlled by using clean seed treated with a suitable fungicide, such as Spergon, planting the seed at wider intervals in the seedbeds, avoiding excessive irrigation and mulching, selecting a well-drained site, and cultivating frequently to provide good soil aeration.

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THE EFFECTS OF CERTAIN NUTRIENT TREATMENTS UPON THE RESISTANCE OF COTTON TO *FUSARIUM VASINFECTION*¹

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INTRODUCTION

The resistance of certain crop plants to various pathogenic soil organisms can be affected by subjecting the host plants to nutritional conditions which influence the physiological responses of the plants. In a recent monograph, Garrett (3) has reviewed various aspects of this question in relation to root disease fungi. Wingard (14) has reviewed comprehensively the literature on general disease resistance in plants; and, more recently, Shear and Wingard (5) have suggested that the greater susceptibility of sweet corn to *Phytophthora stewartii* when grown under conditions of inadequate potassium nutrition may be due to an accumulation in the conducting tissues of nitrates which are favorable for growth of the parasitic bacteria and unfavorable for rapid growth of the corn plant under conditions of potassium deficiency.

The literature on the influence of nutrition on the wilt resistance of cotton has been reviewed by Young (15), who found that applications of potassium fertilizers to Arkansas soils for the correction of potassium deficiency was accompanied by marked reduction in wilt severity. His work was confirmed by Dick and Tisdale (2) in Alabama, who also reported that under some conditions nitrogen fertilizer applications alone could effectively reduce wilt severity but that wilt severity was increased following applications of phosphatic fertilizers. Young and Tharp (16) reported increased wilt severity in Arkansas soils following nitrogenous and phosphatic fertilizers, particularly if the fertilizers were not adequately balanced with respect to potassium. A. L. Smith (6) summarized results of a regional wilt test in 9 states and reported reduced wilt following applications of potassium fertilizers, but stated that varietal resistance to wilt was more important than fertilization so far as yield was concerned. Some of the variations in results were attributed to a combination of wilt and nematode infestation in the field plots observed. In a survey of Texas soils, Taubenhaus and coworkers (8) found a correlation between the presence of *Fusarium* in soils and their acidity, with *Fusarium* more prevalent in acid soils.

In sand culture studies Neal (4) reported that wilt resistance of cotton was not increased when plants received a supplementary solution containing

¹ Contribution of the Department of Botany and Bacteriology, Clemson Agricultural College, in cooperation with the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Technical contribution No. 136, South Carolina Agricultural Experiment Station.

² The author wishes to extend to Dr. G. M. Armstrong and Dr. H. D. Barker his sincere thanks for their cooperation during the progress of these experiments and in the preparation of this manuscript.

calcium nitrate as the source of nitrogen. The basic nutrient solution contained nitrate and ammonium nitrogen in a 7:1 ratio. More recently Tharp and Wadleigh (9, 10) studying wilt resistance of several cotton varieties grown in sand cultures in relation to sources of nitrogen and to relative acidity found a significant interaction between nitrogen source and high pH. There was an increase of wilt resistance at pH 8 with nitrates as the source of nitrogen and a decrease in resistance with ammonia as the source of nitrogen at the same pH, with varietal differences in response. Increases in nitrogen and phosphorus levels resulted in decreased wilt resistance while increases in the potassium level of the solutions increased wilt resistance. Armstrong and Schappelle³ noted that cotton plants grown in a solution in which all the nitrogen was supplied as calcium nitrate were attacked less severely by wilt than plants grown in solutions containing nitrogen from calcium nitrate and ammonium sulphate.

In 1943 and 1944 experiments were conducted by the writer to study in greater detail the responses of a highly wilt-susceptible variety of cotton, Half and Half, to different sources of nitrogen in nutrient solutions at two levels of pH.

MATERIALS AND EXPERIMENTAL METHOD

Pyrex glass jars, of 4½ gal. capacity, 12 in. in diameter and 12 in. in height were coated on the outside with black enamel to exclude light and buried to within a few inches of the top in the soil of the greenhouse benches. Covers were of glazed stoneware or of "masonite hardboard" with 5 two-inch-spaced holes in each cover. Four-hole, flat, split corks were used for supporting the plants in these jars. Sixteen plants were mounted in four corks in each jar and the remaining opening in the jar cover was used for the insertion of a glass tube 25 mm. in diameter for draining and refilling the jars and for a smaller glass tube (6-9 mm. in diameter) through which air for the aeration of the solutions was conducted. Changing of solutions could be accomplished in a few minutes with a minimum of handling and disturbance of plant roots.

Solutions were drained and replaced with new solutions at weekly intervals except at the beginning of the experiments when the plants were small. In 1943 cultures in Series 1 and 2 were aerated several hours daily by bubbling air through them with the aid of a small blower and in 1944 aeration of those of Series 3 was continuous, utilizing air from a storage tank and compressor. Water was added to the solutions from time to time to replace transpiration losses and to avoid excessive changes in concentration of salts in the solutions other than those due to absorption of nutrients by the plants.

Sulphuric-acid-delinted seed dusted with Spergon (tetrachloro para-benzoquinone) were planted in trays of steamed river sand. After 3-4 days the seedlings, selected for uniformity, were transferred to the corks in

³ Unpublished results from the South Carolina Agricultural Experiment Station, 1937.

the jars of nutrient solutions. Growth of seedlings was rapid and in 12-14 days they were at an advanced seedling stage, but squares had not yet appeared. Inoculation with the wilt fungus was made at that time.

The cultures of *Fusarium vasinfectum* used for inoculation were grown according to the technique used by Armstrong (1). Inoculation was accomplished by a 10-minute immersion of the seedling plant roots in a mycelial suspension of the fungus, after which the plants, together with mycelia adhering to the roots, were replaced in their respective nutrient solutions.

There were three jars of each solution and the location of these was randomized so as to permit subsequent statistical analysis. Non-inoculated or check cultures were also grown for each solution.

The molar concentrations of the salts used are presented in table 1. Stock solutions of convenient concentration of all the nutrient salts were prepared and presented no problems, except in the case of calcium sulphate. Stock solutions of this salt, because of its low solubility, had to be prepared in dilute concentration in considerable volume. In mixing the solutions the monohydrogen potassium phosphate was always added last, with stirring, to the full volume of solution; but even with this precaution there was turbidity in the solutions of higher pH, indicating a certain amount of precipitation of the salts. Except for sulphur the molar concentrations of all nutrient elements were the same in the solutions used. Iron was supplied as ferric citrate in a concentration of 2 p.p.m. of ferric iron. Manganese was supplied in a concentration of 1 p.p.m. and copper, zinc and boron in concentrations of 0.5 p.p.m. for each element.

The height of the plants in each jar was measured at the time of inoculation and twice weekly thereafter. After inoculation the plants were inspected daily for the appearance of wilt symptoms. After these had appeared inspections were made twice weekly and the wilt index of the plants was recorded. Normal plants, mildly wilted, moderately wilted, severely wilted, and very severely wilted or dead plants were given values of 0, 1, 2, 3, 4, respectively, which were used to compute the wilt indices. Since 16 plants were grown in each solution culture the greatest possible wilt index for any one solution was 16×4 , or 64. This wilt index was necessarily of an arbitrary nature but it enabled the development of wilt in each jar to be readily followed. Dead plants or plants given a value of 4 were removed from the jars and a portion of the base of the stem cultured in the laboratory to recover the wilt fungus.

Two levels of pH were used in the solutions because of the differences in absorption of ammonium and nitrate ions by plants at different pH levels reported by Tiedjens and Robbins (12). The high pH solutions had an initial pH of 6.6-6.8 and the low pH solutions a pH of 5.0-5.3. In Series 1 and 2 (1943) changes in solution pH were measured from time to time but no attempt was made to maintain the solutions at their initial pH. In Series 3 (1944) an attempt was made to correct wide changes in pH due to

TABLE 1.—*The molar concentrations and pH of the solutions used*

Salt	Low pH			High pH		
	Calcium nitrate nitrogen	Ammo- nium sulphate nitrogen	Ammo- nium nitrate nitrogen	Calcium nitrate nitrogen	Ammo- nium sulphate nitrogen	Ammo- nium nitrate nitrogen
MgSO ₄ · 7H ₂ O	0.002	0.002	0.002	0.002	0.002	0.002
K ₂ SO ₄	0.00075	0.00075	0.00075
KH ₂ PO ₄	0.0025	0.0025	0.0025	0.001	0.001	0.001
K ₂ HPO ₄	0.0015	0.0015	0.0015
(NH ₄) ₂ SO ₄	0.002	0.002
Ca(NO ₃) ₂ · 4H ₂ O	0.002	0.002
NH ₄ NO ₃	0.002	0.002
CaSO ₄ · 2H ₂ O	0.002	0.002	0.002	0.002
Fe, Cu, Mn, Zn, Bo	Trace	Trace	Trace	Trace	Trace	Trace
Initial pH (Series 1, 2, and 3)	5.0	5.3	5.3	6.7	6.8	6.8
Approximate ^a pH (Series 1 and 2) at time of solution change	6.3	3.9	5.2	6.9	4.3	5.5
Approximate ^a pH (Series 3) at time of solution change	5.1	5.2	5.5	6.8	6.0	6.1

^a These pH values are approximations derived from the pH measurements and are indicative of the pH trends observed in these solutions. Close control of the pH was not obtained except for the high pH nitrate solutions.

absorption of nutrients by the plants by the addition of either a dilute sulphuric acid solution or a suspension of calcium hydroxide.

PRESENTATION OF DATA

Table 1 shows the molar concentration of the salts used and the initial pH of each solution. The two lower lines of figures show the trend of pH changes in the solutions of all series. The high pH calcium nitrate solutions did not vary appreciably from their initial pH at any time, although the pH increased slightly from 6.6-6.8 to 6.8-7.0. In Series 1 and 2 where no attempt was made to compensate for changes in solution acidity due to differential absorption of nutrients by the plants the low pH calcium nitrate solutions became less acid. In Series 3 the addition of small amounts of

TABLE 2.—*The number of plants grown in each treatment and the number of plants that escaped infection*

Treatment	Series 1		Series 2		Series 3	
	Total No. of plants	No. of plants not in- fected	Total No. of plants	No. of plants not in- fected	Total No. of plants	No. of plants not in- fected
Low pH (NH ₄) ₂ SO ₄	48	2	47	0	48	0
High pH "	43	0	48	14	42	0
Low pH NH ₄ NO ₃	47	0	48	2	46	0
High pH "	48	6	47	5	48	1
Low pH Ca(NO ₃) ₂	48	8	48	2	48	0
High pH "	47	27	45	11	48	17

dilute sulphuric acid to these solutions kept them near the initial and desired pH. In Series 1 and 2, both high and low pH ammonium sulphate solutions increased in acidity to the point where the growth of the cotton plants was markedly retarded. In Series 3, with excess acidity offset through the use of a calcium hydroxide suspension added from time to time to the solutions, a considerably better growth of plants resulted, although in general vigor and appearance these plants were inferior to those grown

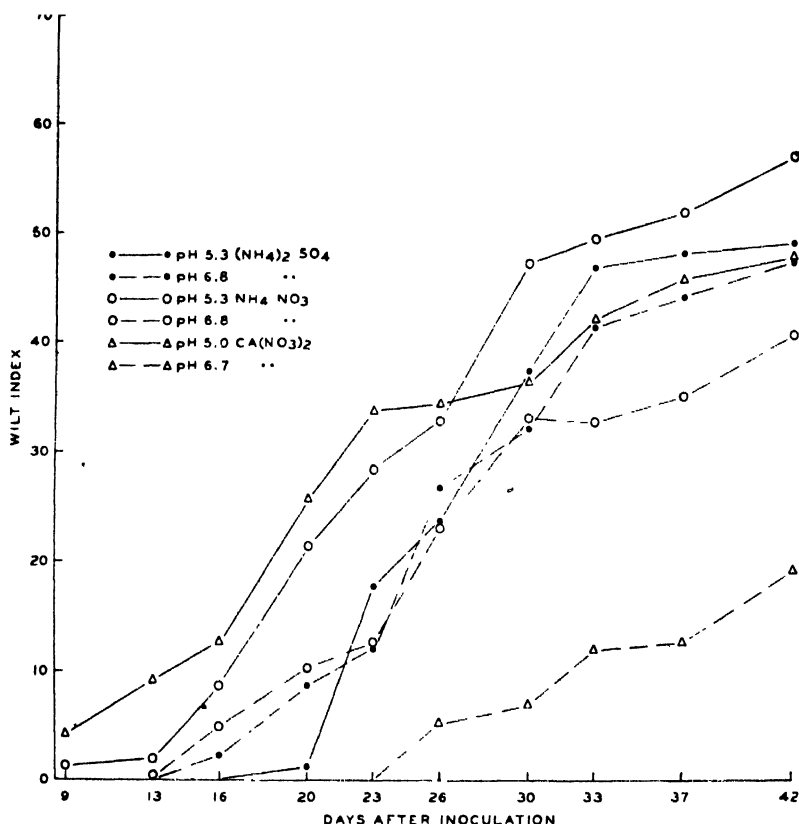


FIG. 1. The mean sum of the wilt indices of the plants in each solution of Series 1 on the indicated number of days after inoculation. Inoculated July 15, 1943.

in calcium nitrate and ammonium nitrate solutions. The pH changes of the ammonium nitrate solutions were intermediate between those of the calcium nitrate and ammonium sulphate solutions. In Series 1 and 2 the high pH ammonium sulphate and ammonium nitrate solutions became more acid and the attempt to correct this acidity in Series 3 was not entirely successful. It should be pointed out that the changes in pH of the solutions presented in table 1, because of the logarithmic character of pH values, are approximations useful in indicating trends rather than representing true means.

The largest and most vigorous noninfected or healthy plants were pro-

duced in the low pH calcium nitrate solutions. Slightly less favorable conditions for growth were present in the high pH calcium nitrate solution and in the ammonium nitrate solutions of both high and low pH. In the ammonium sulphate solutions of Series 1 and 2, in which the pH was 3.8 and 4.5, plant growth was poor, but in Series 3, where excessive acidity was corrected, growth was improved, although markedly less than in the calcium nitrate and ammonium nitrate solutions.

The wilt index values and the percentages of severely wilted or dead

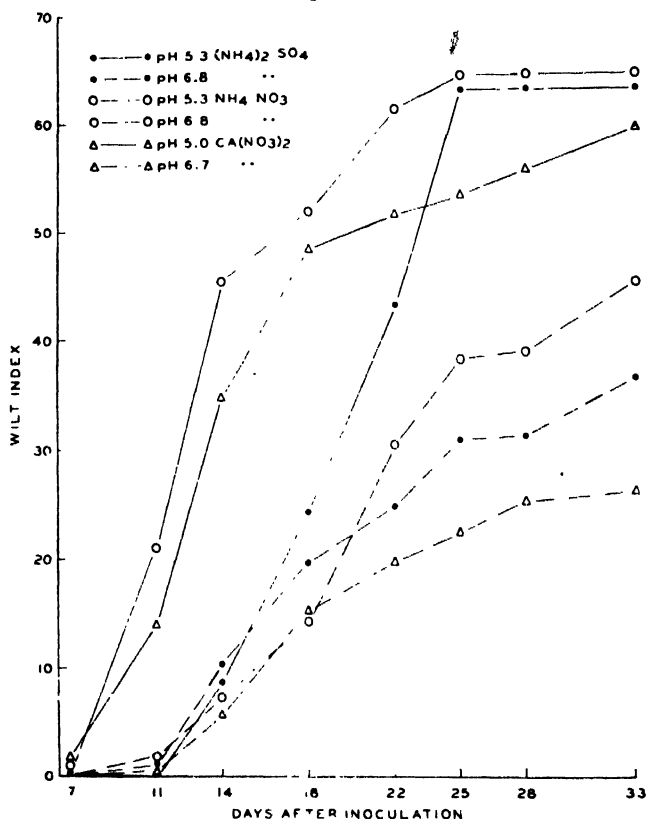


FIG. 2. The mean sum of the wilt indices of the plants in each solution of Series 2 on the indicated number of days after inoculation. Inoculated Sept. 24, 1943.

plants are shown in figures 1, 2, and 3, and figures 4, 5, 6, respectively. Table 2 shows the number of plants of each treatment which had no visible wilt symptoms and from which the *Fusarium* was not recovered on plate cultures at the end of the experiment. The number of plants grown in each solution is also shown in table 2. A considerable number of plants in each series of the high pH calcium nitrate solutions and in the high pH ammonium sulphate solutions of Series 2 apparently did not become infected after exposure to the fungus. The considerable number of noninfected plants in the high pH calcium nitrate solutions of all series indicated that

plants in such solutions were not so readily infected as in other solutions. Some of the plants in the high pH calcium nitrate solutions of Series 3 had blackened vascular tissue although the plants had no wilt symptoms nor could the organism be recovered from plate cultures at the end of the experiment, suggesting that initial infection may have occurred but that the *Fusarium* had not survived. The absence of infection of plants in the high pH ammonium sulphate solutions of Series 2 was probably due to chance since it occurred only in this instance.

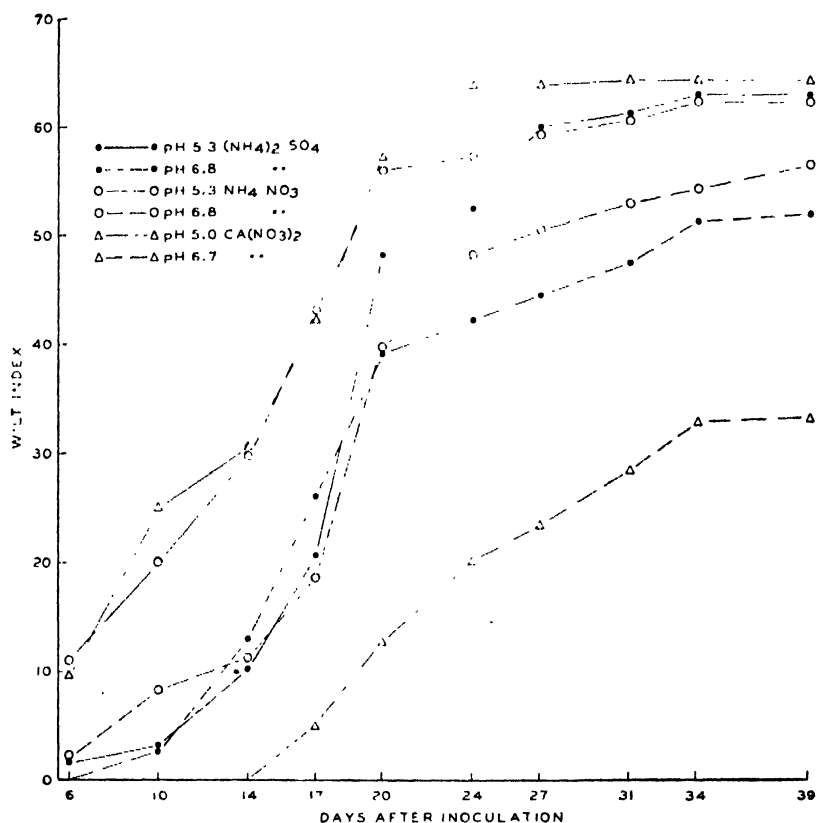


FIG. 3. The mean sum of the wilt indices of the plants in each solution of Series 3 on the indicated number of days after inoculation. Inoculated Aug. 26, 1944.

The single degree of freedom method of analysis described by Snedecor (7) and also discussed by Wadleigh and Tharp (13) and by Tharp, Wadleigh, and Barker (11) in their nutritional studies of cotton, was followed in the statistical comparisons of the data presented in figures 1-6. The general methods followed are illustrated in table 3. The results of individual comparisons are shown in table 4.

DISCUSSION

In general, wilt symptoms began appearing 5-7 days after inoculation and appeared first in low pH calcium nitrate and low pH ammonium nitrate

solutions. In these solutions plant symptoms appeared earlier and wilt development was more rapid than in the ammonium sulphate solutions, but after plants in the latter solutions began to show symptoms of wilt they usually deteriorated quickly. As is shown in figures 1, 2, and 3, the plants in low pH solutions had more pronounced wilt symptoms early in the experiment than plants in high pH solutions, but in time these differences became less, with the exception of the high pH calcium nitrate solutions,

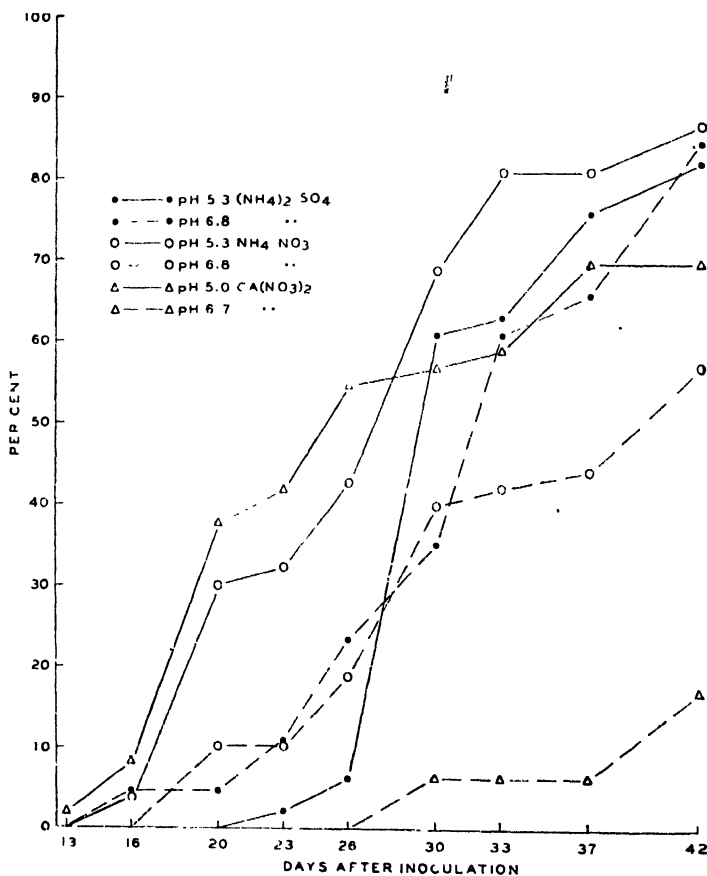


FIG. 4. The percentage of severely wilted and dead plants in cultures of Series 1 on the indicated number of days after inoculation. Inoculated July 15, 1943.

where the appearance of symptoms was delayed and the wilt severity was significantly reduced.

Inspection of figures 1-6 shows that each series of plants, necessarily grown under varying seasonal conditions, showed varying degrees of wilting. The differences between comparisons of treatments in Series 1, as shown in table 4, were less pronounced than those for Series 2 and 3. This is reflected in the smaller number of statistically significant differences in Series 1. The trend, however, was the same as shown by the plus (+) and minus (-) values for each comparison.

Table 4, comparison 1, shows that the plants grown in low pH solutions had a higher wilt index and a higher number of severely wilted and dead plants than the plants grown in high pH solutions, except for the data of August 7. Comparisons 2, 3, and 4 show several differences of probable significance, but they are isolated instances, and do not indicate a consistent trend in the 3 series of plants, and will not be further discussed. Plants grown in low pH calcium nitrate solutions as compared with those grown

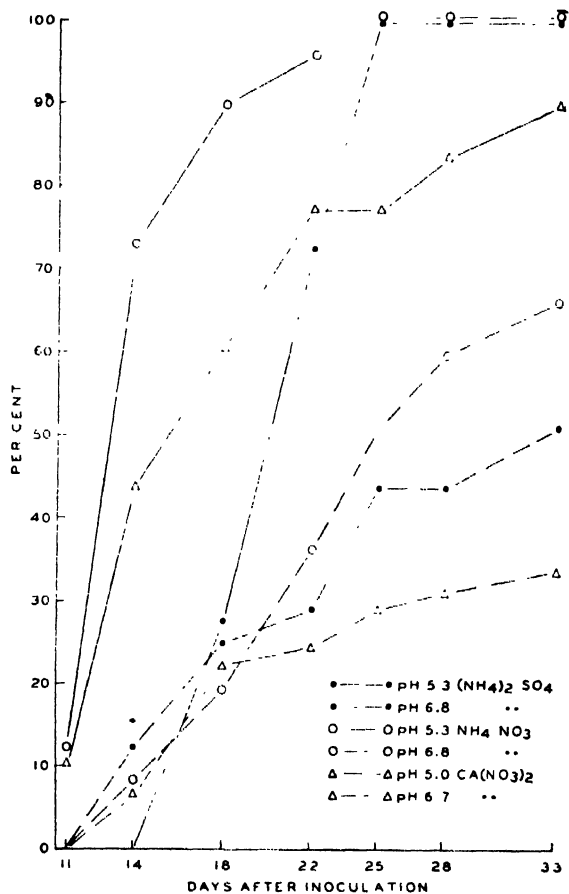


FIG. 5. The percentage of severely wilted and dead plants in cultures of Series 2 on the indicated number of days after inoculation. Inoculated Sept. 24, 1943.

in high pH calcium nitrate solutions (Comparison 5) had a higher wilt index and a greater number wilted severely or dead. These differences were of probable significance for Series 1 (except on Aug. 7), of probable and high significance for Series 2, and of high significance for Series 3. Comparisons 10 and 12 between plants grown in high pH calcium nitrate solutions and low pH ammonium sulphate and ammonium nitrate solutions, respectively, also showed significantly lower wilt indices and a smaller number of severely wilted and dead plants for the high pH calcium nitrate solutions,

except on Aug. 7 in Series 1 and on Oct. 12 in Series 2, comparison 10. The lack of significant differences on Aug. 7 and Oct. 12 was probably due to the slower development of wilt symptoms in ammonium sulphate solutions previously mentioned. There was, then, as shown in comparisons 5, 10, and 12, a consistently lower wilt index and lower wilt mortality in high pH calcium nitrate solutions than in low pH solutions in which the nitrogen was derived from calcium nitrate, ammonium nitrate, and ammonium sulphate.

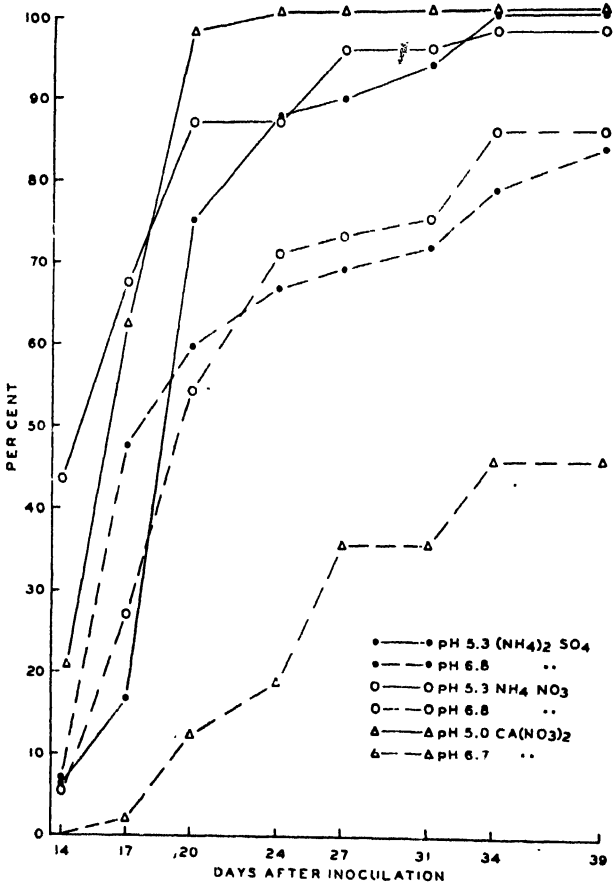


FIG. 6. The percentage of severely wilted and dead plants in cultures of Series 3 on the indicated number of days after inoculation. Inoculated Aug. 26, 1944.

If the wilt indices and the percentage of severely wilted and dead plants in the high pH calcium nitrate solutions are compared with those for the high pH ammonium sulphate solutions, as in comparison 11, it will be noted that in Series 1 and 3 the plants of greater age in the former solution were significantly lower as to both wilt index and the number of severely wilted and dead plants than those in the latter solution. The lack of significant differences in Series 2 was probably due to the relatively large number of

plants in the high pH ammonium sulphate solutions that, for some unknown reason, escaped infection (table 2). Because of the slower development of wilt in plants grown in ammonium sulphate solution, the plants of intermediate age in Series 1, 2, and 3, respectively, did not show significant differences on Aug. 7, Oct. 12, and Sept. 15. In comparison 13, the high pH calcium nitrate plants of Series 3 were significantly lower in wilt index and severely wilted and dead plants than the high pH ammonium nitrate plants, whereas in Series 1 and 2 the differences between these two treatments were not significant. In Series 3 pH changes in the solutions were adjusted so

TABLE 3.—*Analysis of variance of severely wilted and dead plants illustrating several sets of comparisons computed from the same data. Series 2, October 27, 1943*

Group comparison	Treatments	Degrees of freedom	Mean square
	TOTAL	17	1070.257
A	Low pH - High pH	1	9507.745**
	$\text{Ca}(\text{NO}_3)_2 - (\text{NH}_4)_2\text{SO}_4$	1	631.040
	$2[\text{NH}_4\text{NO}_3] - [\text{Ca}(\text{NO}_3)_2 + (\text{NH}_4)_2\text{SO}_4]$	1	813.961
	$(\text{pH} \times \text{Solution}) \text{Ca}(\text{NO}_3)_2 - (\text{NH}_4)_2\text{SO}_4$	1	50.102
	$(\text{Interaction}) 2(\text{NH}_4\text{NO}_3) - [\text{Ca}(\text{NO}_3)_2 + (\text{NH}_4)_2\text{SO}_4]$	1	328.093
	ERROR	12	571.952
B	Low pH - High pH	1	9507.745**
	Low pH $\text{Ca}(\text{NO}_3)_2$ - Low pH $(\text{NH}_4)_2\text{SO}_4$	1	162.760
	High pH $\text{Ca}(\text{NO}_3)_2$ - High pH $(\text{NH}_4)_2\text{SO}_4$	1	518.382
	$2(\text{Low pH NH}_4\text{NO}_3) - [\text{Low pH Ca}(\text{NO}_3)_2 + \text{Low pH } (\text{NH}_4)_2\text{SO}_4]$	1	54.253
	$2(\text{High pH NH}_4\text{NO}_3) - [\text{High pH Ca}(\text{NO}_3)_2 + \text{High pH } (\text{NH}_4)_2\text{SO}_4]$	1	1087.800
	ERROR	12	571.952
C	Low pH $\text{Ca}(\text{NO}_3)_2$ - High pH $\text{Ca}(\text{NO}_3)_2$	1	4719.132*
	Low pH $(\text{NH}_4)_2\text{SO}_4$ - High pH $(\text{NH}_4)_2\text{SO}_4$	1	3444.010*
	Low pH NH_4NO_3 - High pH NH_4NO_3	1	1722.798
	$\text{Ca}(\text{NO}_3)_2 - (\text{NH}_4)_2\text{SO}_4$	1	631.040
	$2(\text{NH}_4\text{NO}_3) - [\text{Ca}(\text{NO}_3)_2 + (\text{NH}_4)_2\text{SO}_4]$	1	813.961
	ERROR	12	571.952

* Indicates significance at the 5 per cent level.

** Indicates significance at the 1 per cent level.

that the pH level was higher than in Series 1 and 2 where the pH decreased between solution renewals. In the higher pH of the solutions of Series 3 wilt susceptibility and wilt mortality increased in the ammonium nitrate solutions.

If the differences discussed in comparisons 1, 11, and 13 were due to the source of nitrogen at the respective pH level of the nutrient solutions used and were not due to the particular balance of the nutrient salts, it follows that two factors, nitrate nitrogen and high pH, both contributed to decreased wilt incidence and plant mortality and that neither factor, by itself, would account for the observed differences. In this connection it has been reported by Taubenhaus and coworkers (8) that wilt was less prevalent in Texas soils of high pH. In regard to the possibility that the response of the plants to inoculation with the wilt organism was a nutrient

TABLE 4.—Comparative effects of pH and nutrients upon the severity of wilt at an intermediate and at the final date of observation of each series

Comparison	Comparison No.	Series 1			Series 2			Series 3 (1944)		
		Aug. 7		Aug. 26	Oct. 12		Oct. 27	Sept. 15		Oct. 4
		Wilt index	Severe wilt and dead		Wilt index	Severe wilt and dead		Wilt index	Severe wilt and dead	
Low pH - High pH	1	+	+	+	+	+	+	+	+	+
$\text{Ca}(\text{NO}_3)_2 - (\text{NH}_4)_2\text{SO}_4$	2
$\text{Ca}(\text{NO}_3)_2 - \text{NH}_4\text{NO}_3$	3
$(\text{NH}_4)_2\text{SO}_4 - \text{NH}_4\text{NO}_3$	4	+	+	+	+	+	+	+	+	+
Low pH $\text{Ca}(\text{NO}_3)_2$ - High pH $\text{Ca}(\text{NO}_3)_2$	5
Low pH $\text{Ca}(\text{NO}_3)_2$ - Low pH $(\text{NH}_4)_2\text{SO}_4$	6
Low pH $\text{Ca}(\text{NO}_3)_2$ - Low pH NH_4NO_3	7
Low pH $\text{Ca}(\text{NO}_3)_2$ - High pH $(\text{NH}_4)_2\text{SO}_4$	8
Low pH $\text{Ca}(\text{NO}_3)_2$ - High pH NH_4NO_3	9
High pH $\text{Ca}(\text{NO}_3)_2$ - Low pH $(\text{NH}_4)_2\text{SO}_4$	10
High pH $\text{Ca}(\text{NO}_3)_2$ - High pH $(\text{NH}_4)_2\text{SO}_4$	11
High pH $\text{Ca}(\text{NO}_3)_2$ - Low pH NH_4NO_3	12
High pH $\text{Ca}(\text{NO}_3)_2$ - High pH NH_4NO_3	13
Low pH $(\text{NH}_4)_2\text{SO}_4$ - High pH $(\text{NH}_4)_2\text{SO}_4$	14
Low pH $(\text{NH}_4)_2\text{SO}_4$ - Low pH NH_4NO_3	15
Low pH $(\text{NH}_4)_2\text{SO}_4$ - High pH NH_4NO_3	16
High pH $(\text{NH}_4)_2\text{SO}_4$ - Low pH NH_4NO_3	17
High pH $(\text{NH}_4)_2\text{SO}_4$ - High pH NH_4NO_3	18
Low pH NH_4NO_3 - High pH NH_4NO_3	19

* Plus and minus signs are used to designate the solution in each comparison in which wilt severity was significantly greater than that observed in the other solution. Plus signs indicate greater wilt severity for the first member of the comparison; minus signs, the second member. A single plus or minus sign indicates significance at the 5 per cent level; double signs, at the 1 per cent level.

balance effect, definite information might be obtained by noting the effects of nitrate nitrogen at a pH of 6.6 to 7.0 over wide ranges of nutrient salt balance. It has been shown, however, that with the balance of nutrients in the solutions used in this investigation a supply of nitrate ions derived from calcium nitrate at a pH of 6.6 to 7.0 together with an absence of ammonium ions markedly influenced wilt susceptibility and plant mortality.

There are several other comparisons in which significant differences were limited to Series 2. Comparisons 14 and 17 show a lower wilt index and less severe wilt for plants in high pH ammonium sulphate solutions, doubtless due to the number of plants in this series that escaped infection. The differences shown in comparisons 6, 8, and 15 are due to the relatively slower development of wilt symptoms in plants grown in ammonium sulphate solutions (observed in all series) which attained statistical significance only in Series 2. In comparisons 9 and 19 only the differences observed on Oct. 12 in Series 2 were due to a delay in appearance of wilt symptoms after inoculation of plants in high pH ammonium nitrate solutions. This delay was also observed in Series 3, but was not statistically significant.

The data indicate that at a pH near neutrality, wilt resistance increased only when nitrogen was derived from calcium nitrate in solutions of the nutrient balance used in these experiments.

SUMMARY

1. Plants grown in high pH calcium nitrate solutions had markedly less wilt injury and a lower mortality than those grown in any other solution.
2. On plants grown in low pH calcium nitrate solutions severe wilt symptoms developed in a shorter time than in any other solution.
3. Severe wilt injury developed rapidly on plants in low pH ammonium nitrate solutions.
4. Wilt injury in the high pH ammonium nitrate solutions was less than in the low pH ammonium nitrate solutions but was greater than in the high pH calcium nitrate solutions.
5. Wilt injury developed more slowly on plants grown in solutions containing only nitrogen from ammonium sulphate than on those grown in low pH calcium nitrate or ammonium nitrate solutions.
6. Two mutually dependent factors, a pH of 6.6–7.0 and nitrate nitrogen derived from calcium nitrate at the particular nutrient balance used, contributed significantly to increased wilt resistance and decreased wilt injury.

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ATTEMPTS TO CONTROL BACTERIAL BLIGHTS OF PEAR AND WALNUT WITH PENICILLIN

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It is well known that penicillin, primarily bacteriostatic against Gram-positive bacteria, may also inhibit the growth of certain Gram-negative organisms. Brown and Boyle¹ reported that the penicillin sensitivity of the Gram-negative organism of crown gall, *Agrobacterium tumefaciens* (Smith and Townsend) Conn² syn. *Phytomonas tumefaciens* Bergey *et al.* differed little, if at all, from that of *Micrococcus pyogenes* var. *aureus* (Rosenbach) Zoph. syn. *Staphylococcus aureus* Rosenbach, the organism generally used to determine the assay value of penicillin. But they themselves point out that the conditions under which their experiments were conducted may leave the results open to question. They also found weak solutions of penicillin, even in an unpurified state, to be distinctly effective *in vivo* against crown gall on *Bryophyllum* when the galls were wrapped with cotton soaked in the penicillin solution and punctured with needles pushed through the wet cotton.^{1, 3} In earlier studies by Brown and Boyle penicillin was effective *in vitro* against *Erwinia carnegiana* Standring, the Gram-positive pathogen responsible for the rot of the giant cactus (*Carnegieana gigantea* Britt. and Rose) in the Pacific Southwest. They also obtained some evidence of susceptibility of *Corynebacterium sepedonicum* (Spieckermann and Kothoff) Skaptason and Burkholder, the potato-ring-rot pathogen, to penicillin *in vitro*.⁴ Waksman^{5, 6} and his associates also have contributed additional information on the effect of several antibiotics on various plant pathogens.

EXPERIMENTS AT THE DECIDUOUS FRUIT FIELD STATION

In the fall of 1944, experiments were undertaken to test the antibiotic activity of unpurified penicillin against two important Gram-negative plant pathogens, namely *Xanthomonas juglandis* (Pierce) Dowson, syn. *Phytomonas juglandis* Bergey *et al.* [walnut blight] and *Erwinia amylovora*

¹ Brown, J. G., and Alice M. Boyle. Application of penicillin to crown gall. *Phytopath.* 35: 521-524. 1945.

² The nomenclature for all bacteria referred to in this paper is that used in the sixth edition of Bergey's Manual of Determinative Bacteriology now in press, and was kindly furnished by Dr. Robert S. Breed, Chairman, Board of Editors, New York State Experiment Station, Geneva, N. Y. If the nomenclature is being used in the sixth edition of the Manual for the first time, the latest accepted synonym used in the fifth edition is also given in this paper.

³ Brown, J. G., and Alice M. Boyle. Penicillin treatment of crown gall. *Science* 100: 528. 1944.

⁴ Brown, J. G., and Alice M. Boyle. Effect of penicillin on a plant pathogen. *Phytopath.* 34: 760-761. 1944.

⁵ Waksman, Selman A. Microbial antagonisms and antibiotic substances. 350 pp. New York Commonwealth Fund. 1945.

⁶ Waksman, Selman A., Elizabeth Bugie, and H. Christine Reilly. Bacteriostatic and bactericidal properties of antibiotic substances, with special reference to plant-pathogenic bacteria. *Bul. Torrey Bot. Club* 71: 107-121. 1944.

(Burrill) Bergey *et al.* [pear blight]. An improved strain of *Penicillium notatum* (1249-b-21), originally obtained from the Northern Regional Research Laboratory, Peoria, Illinois, was grown upon a modified Czapek-Dox liquid medium supplemented with lactose and corn steep liquor. The flat-bottle method was employed, and all known requirements for the maximum production of penicillin with regards to the length of time for cultivating the fungus, the pH, temperature, etc., were observed. In replicated tests the assays were sufficiently high to markedly inhibit growth of *Micrococcus pyogenes* var. *aureus*. The strain used was obtained from the Food and Drug Administration of the Federal Security Agency and was one developed by them for assay purposes.

Dr. C. E. Clifton⁷ and his associates devised a convenient assay method, not heretofore reported, which obviates the use of glass assay cylinders or paper discs. Briefly the method is as follows: exactly 30 ml. of a suitable 2 per cent nutrient agar are poured into standard Petri plates of 100-mm. diameter and seeded in any one of the several conventional ways with 0.1 ml. of a 1 to 10 dilution of a 24-hour culture of *Micrococcus pyogenes* var. *aureus*. Holes exactly 10 mm. in diameter are cut in the seeded medium with any sharp-edge, sterile, metal tube, such as a cork borer. Into a hole is dropped 0.1 ml. of the penicillin-containing broth to be assayed, after which the plates are incubated for 24 hours at 35–37° C., the optimum temperature for growth of the organism. The number of units of penicillin per milliliter of the test solution is determined by referring the measurement of the diameter of the circular area of inhibition in the plate to a table of known value. *Xanthomonas juglandis* tested against the unpurified penicillin in this manner seemed to be inhibited to a slight extent, but the growth of *Erwinia amylovora* was not affected. Both of these organisms were incubated at 30° C., the temperature generally considered to be their optimum. Also, cultures of *Xanthomonas juglandis*, somewhat older than 24 hours and with maximum turbidity, were used to seed the plates, since this organism grows rather slowly even at its optimum temperature.

This method also permits of determining the number of units of commercial penicillin required to bring about complete inhibition of the growth of any susceptible organism by introducing graduated dilutions of the drug, the assay value of which is known, into the plates seeded with the organism.

Antibiotic Activity of Commercial Penicillin Against Erwinia amylovora in Vitro. Experiments with unpurified penicillin were discontinued when public sale of the pure, commercially prepared drug was permitted by government directive on March 15, 1945. Soon after that date the writer entered into a cooperative arrangement with the Commercial Solvents Corporation of Terre Haute, Indiana, which supplied generous quantities of pure penicillin of known assay value for additional studies, and for which grateful acknowledgment is made here.

⁷ The writer is deeply indebted to Dr. Clifton, Department of Bacteriology, Stanford University, for many helpful suggestions and criticisms during the course of the work and in the correction of the manuscript.

Strains of *Erwinia amylovora* from different sources were tested against graduated dilutions of commercial penicillin in sterile, distilled water by the plate method described. Certain autogenous cultures from pear trees and others from apple trees in the same orchard were sensitive to the penicillin; all other cultures were resistant to its action. The circular areas of inhibition in the plates were clean-cut, but all measurements were made under the microscope when it was observed that minute colonies of the organism, imperceptible to the naked eye, slowly grew in a narrow zone, usually about 5 mm. wide, between the area of absolute inhibition and that of unrestricted growth. Under the microscope, the organism growing in these marginal zones appeared either as tiny, discrete colonies or as scarcely more than a granulation in the agar. Throughout this paper the diameter of the zones of inhibition as determined by both microscopic and macroscopic inspection are given, the first figure representing the diameter as observed under the microscope and the second as measured directly with the naked eye.

Dilutions of commercial penicillin containing 1000 units per 0.1 ml. inhibited the growth of the autogenous strains of *Erwinia amylovora* in circular areas of 35 to 45 mm. diameter. Dilutions of 100 units per 0.1 ml. inhibited growth in circular areas varying from 25 to 35 mm. Dilutions of 10 units per 0.1 ml. failed to affect the organism at all.

In parallel experiments with *Erwinia amylovora* from apple tested against the same lot of penicillin, a slightly greater resistance to the drug was noted; thus dilutions of 100 units per 0.1 ml. inhibited growth in circular areas of only 20 to 30 mm. diameter.

In check plates of *Micrococcus pyogenes* var. *aureus*, dilutions of the same lot of penicillin containing only 0.1 unit per 0.1 ml. inhibited growth in circular areas with diameters varying from 25 to 35 mm.

Antibiotic Activity of Commercial Penicillin Against Xanthomonas juglandis in Vitro. The walnut-blight organism proved to be definitely more susceptible to penicillin than *Erwinia amylovora*. Whereas a dilution of the drug containing 10 units per 0.1 ml. failed to affect the *Erwinia*, the growth of *Xanthomonas juglandis* in replicated tests was inhibited in circular areas of 20 to 30 mm. diameter. At dilutions of 1 unit per 0.1 ml. the organism was not affected.

That penicillin is not only bacteriostatic in its action against *Xanthomonas juglandis* and *Erwinia amylovora*, but bactericidal as well, is evidenced by the fact that no colonies of the organism ever developed in the circular areas of complete inhibition regardless of time. Numerous subcultures made at frequent intervals for two weeks from such areas in plates of *Xanthomonas juglandis* remained sterile.

Attempts to Control Erwinia amylovora in Vivo. Four mature Bartlett pear trees severely affected with blight were injected with penicillin solution in June, 1945. Infection had taken place several months earlier, almost exclusively through the blossoms as is characteristic of the disease in the Santa Clara Valley, and had spread to the twigs and limbs where it was

still very active. Four holes, $\frac{7}{16}$ inches in diameter and not less than $3\frac{1}{2}$ inches deep, were bored just below the crotch of a tree and equidistant on the circumference of the trunk which averaged 22 inches. Pieces of galvanized iron tubing, $\frac{7}{16}$ inches in diameter and $2\frac{1}{2}$ inches long, were driven just deep enough into the holes to insure a tight seal. Five-foot lengths of rubber hose attached to the projecting ends of the tubes conveyed the penicillin solution in a straight drop from clean cans suspended in the trees. The cans were ventilated through cotton filters in the lids.

One tree was injected with 500 ml. of a solution of commercial penicillin containing slightly in excess of 1000 units per ml. It was this dilution, 0.1 ml. of which inhibited growth of *Erwinia amylovora* in circular areas 25 to 35 mm. in diameter in test plates. The drug was dissolved in distilled water near the freezing point to offset possible deterioration by the warm atmospheric temperatures prevailing at the time. The three other trees received identical dilutions of the drug prepared in the same way, but only 250 ml. of solution per tree were used.

The trees showed no unfavorable reaction to the drug. The solution was readily absorbed by all four trees within a few hours, but the results were unqualified failures. A day after injecting the trees, bits of diseased wood from twigs selected as close to the point of injection as possible, were planted in sterile, green, pear-fruit tissue and incubated at 30° C. *Erwinia amylovora* grew luxuriantly in practically 100 per cent of the cultures prepared in this way.

A fifth tree received protracted treatment with a stronger solution containing 1408 units of commercial penicillin per ml. The drug was pure, but, unlike the commercial product, it had not gone through the final process of sterilization. In this condition it is much cheaper but is considered unsuited for therapeutic use in humans or animals. The tree was injected three times at intervals of 24 hours, each time with 500 ml. of the solution. Following the injections, several hours after the solution had been absorbed, one liter of sterile, distilled water was added to the can for the sole purpose of keeping the wood in an absorptive condition, experience obtained in preliminary experiments having shown that if considerable time is allowed to elapse between injections the wood ceases to absorb the solution. This may possibly be due to the formation of tyloses or the production of gum in the tracheae.

The result of this experiment was an unqualified failure. Actually more of the numerous cultures made from the treated wood yielded the organism than did wood from cheek trees adjoining. This must be regarded as a coincidence.

Attempts to Control Xanthomonas juglandis in Vivo. In April, 1945, a total of 11 mature Payne (English) walnut trees with an average diameter of 29 inches growing near the Deciduous Fruit Field Station were injected with commercial penicillin in solution prepared in precisely the same way as described above. No less than 4 and usually 8 injections were made per tree depending upon the circumference of the trunks.

Plate tests reported elsewhere in this paper showed that 0.1 ml. of a dilution of the drug containing 100 units per ml. was sufficient to inhibit growth of *Xanthomonas juglandis* in large areas. The strength of the solution used in the trees varied from 200 to 600 units per ml., and each tree received 1 liter of solution.

The results were unqualified failures. Broth cultures made from occasional lesions on the petioles of leaves taken as close to the points of injection as possible yielded the organism readily.

The Effect of Passage Through Pear Wood on Penicillin. To determine whether the failure of penicillin to control *Erwinia amylovora* *in vivo* might have been due to adsorption or inactivation of the drug by the wood in which it was injected, the following experiment was performed.

A healthy, 3-year-old pear shoot, 1 inch in diameter and 32 inches long, was cut from a dormant tree in January and painted with melted paraffin except at the cut ends. The shoot was then forced through 2 large, perforated rubber corks, one of which served to seal about 10 inches of the shoot inside a Martin vacuum jar. The cork at the opposite end of the shoot fitted into a receptacle holding 20 ml. penicillin solution in which 200,000 units of the drug were dissolved. The Martin jar was evacuated under low vacuum, and the liquid that was extracted from the end of the shoot was removed from time to time. When the last of the penicillin solution had disappeared into the shoot, the pump was kept running for several minutes, and then an indeterminate amount of distilled water was added to the receptacle and drawn through the wood by vacuum.

At the beginning of the experiment, pure, colorless sap started to drip from the end of the shoot in the Martin jar almost as soon as the vacuum was applied. After 25 ml. were collected, a faint suggestion of yellow color in the liquid indicated that some penicillin was entering the sap stream. This extract was discarded and the vacuum pump started again. By the time an additional 50 ml. of liquid were collected, the color of the solution dripping from the shoot was practically the same as that of the original solution, a sample of which had been kept. This extract was also discarded. The pump was started again and 20 ml. of liquid extracted, the deep yellow color of which was only slightly lighter than that of the original penicillin solution. This liquid was used in the following experiments.

A series of 1 to 10 dilutions were made from each of the following: (1) the original penicillin solution; (2) the dark yellow extract from the wood; (3) the same extract from wood after it had been filtered under vacuum through a short No. 3 Chamberland candle. The antibiotic activity of the three series of dilutions was tested at the optimum temperature of the organisms in plates seeded with *Micrococcus pyogenes* var. *aureus* and *Xanthomonas juglandis*.

The number of units of penicillin present was known for only the first of the three series of dilutions, namely that made from the original, unfiltered penicillin solution. This series of 1 to 10 dilutions contained 10,000;

1000; 100; 10 units per ml. A parallel series of dilutions made of the yellow extract from wood had somewhat less antibiotic activity against *Micrococcus pyogenes* var. *aureus* in test plates than the original unfiltered penicillin series. But the difference seemed entirely a matter of dilution rather than inactivation of the drug. Thus, 0.1 ml. of the yellow extract just as it came from the wood inhibited growth of the bacteria in circular areas of 60–70 mm. diameter. As the amount of penicillin decreased with each succeeding dilution, the areas of inhibition, always sharply defined, grew correspondingly smaller. Nevertheless, even the fourth dilution in the series inhibited growth in clean-cut areas of 20–30 mm. diameter.

Passage of the yellow extract from the wood through the Chamberland candle reduced its antibiotic properties still further. Before passage through the candle, the undiluted extract inhibited growth of *Micrococcus pyogenes* var. *aureus* in circular areas of 60–70 mm. diameter. After filtration the diameters of the areas of inhibition were only 46–60 mm.

Growth of *Xanthomonas juglandis* was inhibited in clean-cut, circular areas of 30–40 mm. diameter by the extract as it came from the wood. But a 1 to 10 dilution of the extract failed to affect the bacteria at all except at the edges of the holes in the plates. At these points the growth of the colonies was feeble, and they remained very small.

DISCUSSION

There can be no doubt whatever that growth of certain strains of the Gram-negative organisms *Erwinia amylovora* and *Xanthomonas juglandis* can be inhibited *in vitro* by penicillin if strong enough solutions be used. That the action of the drug is not only bacteriostatic but bactericidal as well was established. The fact that in preliminary phases of the work certain strains of *Erwinia amylovora* proved resistant to strong concentration of penicillin might be attributed to the use of penicillin material that had deteriorated somewhat; before arrangements were completed with the Commercial Solvents Corporation for supplies of the drug, a certain amount of it was purchased in local drug stores. The conditions under which it was kept before purchase were not known, but it is recognized that penicillin slowly deteriorates with age even under the best conditions of storage and rapidly under improper conditions, especially, with improper temperature.

There are several possible reasons why penicillin failed utterly to control *Erwinia amylovora* and *Xanthomonas juglandis* *in vivo*: (1) too small amounts of penicillin solution may have been used, even though its strength was always considerably in excess of the concentrations required to destroy either organism *in vitro*; (2) the drug may have been diluted so much by the sap of the trees in which it was injected that it was rendered harmless; (3) the penicillin may have failed to reach the bacteria in the disorganized tissues of the lesions; (4) more injections at shorter intervals may have been necessary; (5) the action of penicillin may be evident only when in contact with susceptible, actively growing bacteria over a period of time.

With reference to the first reason cited, namely, that too small amounts of the drug may have been injected, it should be stated that at no time was the disinfection of an entire tree expected or contemplated. The high price of penicillin makes experiments of such scope impractical. Even its cost in the small scale tests was prohibitive from the growers' standpoint. For instance, the retail cost of penicillin at the time was \$3.50 per 100,000 units. The cost of injecting 1 walnut tree experimentally with 600,000 units was \$21.00 for the drug alone. The retail price has steadily dropped since then, and at the time of writing this paper penicillin sells for \$1.25 per 100,000 units. Even this price makes its use impractical by growers except in amounts even smaller than those used unsuccessfully in the experiments reported.

It was hoped that the drug might reach and permeate the diseased tissue of some lesions comparatively close to the points of injection in sufficient concentration to kill the bacteria in them. Had this been accomplished, the method of control on a greater scale could be referred to that probable, future day when the cost of the drug, even in large amounts, would be no deterrent to its use.

That the penicillin probably did not reach the bacteria in sufficient concentration to kill them is indicated by the fact that a pear shoot 32 inches long and 1 inch wide yielded 25 ml. of pure sap to begin with, then 50 ml. of sap containing penicillin in ever-increasing amounts but never as much as in the original solution. Actually the original solution (20 ml.) came through the wood largely as a unit; the color of a final 20 ml. of extract indicated this, and its antibiotic activity in plate tests further substantiated it. It is clear that the total sap content of the pear shoot amounted to well over 3 times the original volume of penicillin solution used, a factor that probably entered in the injection experiments and vitiated them. Whereas, the penicillin solution could be pulled through the wood by vacuum largely as a unit in a few minutes, a matter of hours was required to inject a tree during which time the penicillin solution, gradually entering the sap stream, probably was diluted to a point of ineffectiveness.

Logically, for more precise results, the injections should have been made in one of the many scaffold branches of a tree rather than in its trunk, the considerably greater size of which made proportionally greater dilution of the drug injected into it inevitable. Better still, the drug might have been injected into a single diseased twig or shoot or even pulled through one by vacuum, after which cultures made from the wood would have revealed whether the drug had killed the organism or not. But, results obtained in these ways would have little practical application regardless of their possible success. From the beginning it was recognized that control of the two organisms *in vivo*, to have any practical significance, would have to be through the tree trunks. To attempt to inject individual scaffold branches every year would be impractical for growers. Aside from the great amount of time, labor, and equipment that would be required to treat an orchard in

this way, the problem of keeping the wounds sterile until healed to prevent wood decays, by no means simple even when the trunks alone are injected, would become too greatly complicated were it necessary to bore all the scaffold branches with holes. A considerable acreage of pears growing on calcareous soils in the Santa Clara Valley must, for best results, be injected with iron salts every two or three years to correct lime-induced chlorosis. The injections are made in the trunks. Heart rots following such treatment are by no means uncommon. The expansion and contraction of the wood under fluctuating temperatures, as well as the movement of the trees in the wind alone might be sufficient to break the seal between the wood and the wax with which the holes are usually plugged and permit organisms of decay to make their way inside. These same factors would probably make it even more difficult to keep the wounds in scaffold branches sterile until healing was complete.

Whether the diseased wood, unlike healthy wood, resisted passage of the penicillin through it or adsorbed or inactivated the drug is not known. Nor is there any assurance that more injections at shorter intervals might not have produced better results, but again the problem of time, labor, and expense from the growers' standpoint, discouraged the undertaking of such experiments. Lastly, whether the penicillin remained in contact with the organisms long enough to kill them is problematical.

SUMMARY

The antibiotic properties of penicillin were tested against the Gram-negative organisms, *Erwinia amylovora* (Burrill) Bergey *et al.* [pear blight] and *Xanthomonas juglandis* (Pierce) Dawson [walnut blight] *in vitro* and *in vivo*. In conventional plate tests, dilutions of the drug in distilled water at the rate of 100 units per 0.1 ml. inhibited growth of *Erwinia amylovora* completely in circular areas of 25 mm. diameter, as seen under the microscope, or 35 mm., as seen by the naked eye. At the rate of 10 units per 0.1 ml., the organism was not affected. *Xanthomonas juglandis* is more susceptible and was inhibited by dilutions of 10 units per 0.1 ml. in circular areas of 20 to 30 mm. diameter, but it was not affected by 1 unit per 0.1 ml.

Penicillin is not only bacteriostatic but bactericidal as well.

Attempts to control either organism *in vivo* failed completely. Individual pear trees, severely affected with blight, were injected once in their trunks with as much as 500 ml. of penicillin solution containing more than 1000 units per ml. Also, one tree was injected three times, at 24-hour intervals, with 500 ml. penicillin solution containing 1408 units per ml. Numerous cultures made at intervals from active lesions on twigs close to the points of injection showed that no control of the organism had been obtained.

Individual walnut trees were injected with 1 liter of penicillin solution containing 200 to 600 units per ml. with no better success.

To determine whether the penicillin solution might have been adsorbed

or inactivated by the wood, 20 ml. of solution containing 200,000 units were drawn through a 32-inch long, healthy, 3-year-old pear shoot under vacuum. The wood yielded practically 75 ml. of sap before the penicillin solution came through. Finally 20 ml. of extract were collected, the color of which was only slightly lighter than that of the original solution. Its antibiotic properties against *Micrococcus pyogenes* var. *aureus* and *Xanthomonas juglandis* were found to be slightly impaired, but by dilution rather than inactivation. Passage of the extract through a short No. 3 Chamberland candle reduced its antibiotic activity slightly.

The failure of penicillin to control *Erwinia amylovora* and *Xanthomonas juglandis* *in vivo*, even at short distances from points of injection in the wood, is believed to have been due largely to excessive dilution of the drug by the sap. Other possible reasons for the failure are discussed.

The present prohibitive cost of penicillin makes it impractical to use larger amounts or stronger solutions of it than reported, and even if either organism had been controlled *in vivo*, growers still would have to wait until the drug is considerably cheaper before such a method of control could be utilized economically.

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A STUDY OF VEGETABLE SEED PROTECTANTS¹

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Chemical treatment of vegetable seeds to protect against the ravages of damping-off has been the subject of much investigation during the past two decades. While a considerable amount of research had been done earlier, the introduction of the practice of dust treatment of vegetable seeds in the late twenties stimulated greater activity.

Under the stress of war-imposed scarcities of certain metallic salts heretofore principally used, a study of less-scarce metals and of certain newly developed organic fungicides was undertaken in the spring of 1942. Results obtained in the preliminary trials conducted by the junior author prior to his entrance into the armed services led to the detailed study of a few materials by the senior author during the winter of 1942-43. The studies herein reported are additional to tests conducted in cooperation with the committee of the American Phytopathological Society on coordination in cereal and vegetable seed treatment research.

THE DEVELOPMENT OF DUST MATERIALS AS SEED PROTECTANTS

Organic mercury compounds, already in use for a decade in liquid form, were developed for use as dust treatments on vegetable seeds in the mid-twenties. In the United States, by 1926-27, Orton (31), Coors and Stewart (8), Jones (25), and Haenseler (14) were able to report success with these dust materials as seed protectants on vegetables. Although McWhorter (30) found the dust treatment less effective than the comparable liquid treatments in protecting beets against soil-borne pathogens in greenhouse trials, the development of suitable seed protectants in dust form constituted an immense step toward wide adoption of the practice of protective seed treatment. In most cases satisfactorily replacing the more inconvenient method of soaking the seed in liquid solutions, they proved a boon in the struggle against damping-off.

The soaking of vegetable seeds in solutions of copper sulphate had long been practiced with good results by some growers. The ease of application of dusts to the dry seed led Coors and Stewart (8) and Horsfall (17, 18) to test dusting with powdered copper sulphate and monohydrated copper sulphate as a substitute for the method of soaking the seeds in copper sulphate solution. These copper salts were found to adhere poorly to the seed coat and proved to be rather unsatisfactory in performance. However, recognition of the poor adherence as a probable cause of the poor protection stimulated a survey of the other salts of copper, from among which Horsfall (18,

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19) selected red cuprous oxide as being the most promising. Subsequent work by Horsfall (20, 21, 22, 23) and by others (3, 5, 6, 7, 26, 33) has shown the benefits to be derived from the use of this material and also has revealed certain difficulties arising from its use. It has proved to be injurious on many acid soils and, in common with other copper salts, it frequently causes a hardening of the seed coats of lima beans. It is also injurious to crucifers. To reduce interfacial friction between seeds, it has been found necessary to add graphite with the red cuprous oxide (4, 5, 22), a practice which Hulbert and Whitney (24) had found efficacious in reducing interfacial friction between pea seeds treated with Ceresan or with copper carbonate.

A later development was use of the yellow form of cuprous oxide, the color change being the result of reduction in particle size. The yellow cuprous oxide has been more effective than the red form at equal dosages and has been equally effective at reduced dosages (16, 22).

Zinc oxide was found effective as a seed treatment material by Horsfall (20, 21) and was popularized for use on spinach by Cook and Callenbach (5, 6, 7). Other than on spinach and cabbage it has been used to a much less extent than has cuprous oxide. Treatment with zinc oxide also increases the friction between seeds, thus necessitating the addition of graphite where the seeds are to be machine-planted.

The adoption of the oxides of these two metals as seed-protectant materials did much to popularize the practice of treatment of vegetable seed. These materials, relatively inexpensive and effective on many crops and under many conditions, were adopted quickly by commercial vegetable producers. In 1935 the red cuprous oxide treatment was applied to 20,000 bushels of peas in New York (1), and in 1936 it was used on 50,000 bushels in that State alone (2). By 1937 Cook (5) reported that approximately 150,000 pounds of spinach seed were treated annually in the Norfolk, Virginia, area. Because of the popularity of the zinc oxide seed treatment in that area, presumably most of this seed was treated with zinc oxide. During the same period, these materials were accepted and recommended as seed protectants in many states and on many crops.

These developments in the field of protective seed treatments stimulated active search for other effective materials. In 1940 Cunningham and Sharvelle (9) reported promising results obtained in the treatment of lima beans with two synthetic organic compounds. One of these, later identified as tetrachloro-para-benzoquinone and marketed as Spergon (36), has proven generally to be a very satisfactory seed protectant on vegetable seeds. It has been especially successful with legumes (9, 11, 27, 36). Increased height of peas from Spergon-treated seed was observed by Felix (11). Sharvelle and Shema (35) reported evidence of stimulation in field-grown peas while McNew (28) obtained increased size of plants from disease-free peas planted in steamed soil when Spergon was used as the seed treatment material. Davis and Haenseler (10) and Gould (12) reported that, while Spergon was satisfactory with beans, lima beans, and peas, it was less effective than certain

other materials with beets and spinach. Person and Chilton (32) reported that, with flowers, emergence was satisfactory following the use of Spergon but that the final stand was lower than where yellow cuprous oxide was used.

Recognized slightly later than Spergon, two derivatives of carbamic acid, tetramethyl thiuramdisulphide and ferric dimethyldithiocarbamate, have given favorable results in studies reported on in 1943 by McNew (29), Taylor, Leach, and Rupert (37), Davis and Haenseler (10), Gould (12), and Porter (34). Many investigators have reported on these materials since 1943. During 1943 tetramethyl thiuramdisulphide was introduced as a seed-protectant material under the name "Arasan," this containing 50 per cent of the active ingredient in a non-wettable diluent. Ferric dimethyldithiocarbamate, introduced into commercial use as "Fermate," has found its chief value as a protective spray fungicide, though it is, in many cases, an effective seed protectant.

DAMPING-OFF

Throughout these studies the term damping-off has been construed as applying to the rotting of viable seeds and to the killing of the seedlings before emergence, as well as to the more commonly recognized killing of the emerged seedlings. For convenience, the terminology of Horsfall (18) has been employed to distinguish between the types of damping-off.

Pre-emergence damping-off. With this type of damping-off the young seedling is invaded and destroyed before it emerges above the surface of the soil.

Post-emergence damping-off. This is the more commonly recognized stage of damping-off. The already emerged seedling is invaded and the stem becomes water-soaked near the ground line—the plant then topples over suddenly, usually while the cotyledons are still green. With slightly older seedlings the plant occasionally survives invasion but the stem becomes discolored and hard, the so-called wire-stem of seedlings. In other cases small brown lesions may develop on the stem or roots but the seedlings survive the invasion.

The fungus *Pythium ultimum* Trow principally was responsible for the damping-off reported in the following experiments.

MATERIALS

The preliminary trials were with proprietary materials developed as seed protectants, various inorganic salts, and a few organic compounds available from fruit spray experiments. Later, other materials were obtained specifically for trial as seed protectants. All proprietary compounds were provided gratuitously by the several manufacturing companies. A list of the materials tested, together with their composition and source follows:

AAZ: Special zinc oxide; Rohm & Haas Company.

Arasan: Tetramethyl thiuramdisulphide, 50 per cent active material, non-wettable; E. I. duPont de Nemours & Company.

C-119: Copper trichlorophenate, 20 per cent; Dow Chemical Company.

Cuprocide: Red cuprous oxide; Rohm & Haas Company.

Fermate: Ferric dimethyldithiocarbamate, 70 per cent active material, wettable; E. I. duPont de Nemours & Company.

Iron sulphate; Mellon Institute.

Japanese Beetle Spray: Tetramethyl thiuramdisulphide, 80 per cent active material, wettable; E. I. duPont de Nemours & Company.

Phenothiazine, micronized; E. I. duPont de Nemours & Company.

Semesan: Hydroxymercurichlorophenol, 30 per cent; E. I. duPont de Nemours & Company.

Spergon: Tetrachloro-para-benzoquinone; Naugatuck Chem. Div., U. S. Rubber Co.

Tetrachlororesorcinol; Pennsylvania Salt Company.

Thiosan (now Tersan): Tetramethyl thiuramdisulphide, 50 per cent active material, wettable; E. I. duPont de Nemours & Company.

In addition to the above, boric acid, copper carbonate, silver carbonate, silver chloride, silver chromate, silver iodide, silver nitrate, silver orthophosphate, silver oxide, and silver sulphate, C.P. grade, were tested.

PRELIMINARY TRIALS

In a preliminary survey in 1942 the protective value of a number of materials was tested on spinach seeds in one or more of four greenhouse

TABLE 1.—*Preliminary trials on seed treatment of spinach, 100 seeds per plot, with dosage at full maximum capacity of seeds*

	Emergence of plants, in per cent			
	Test 1	Test 2	Test 3	Test 4
Date planted	Apr. 26	May 15	June 4	June 29
Record, days after planting	8	7	6	7
Number of replications	5	4	5	5
Material:				
Silver carbonate	30.0 ^a	60.0	11.0	41.6
Silver chloride		31.8 ^a		
Do		39.0 ^b		
Silver iodide		28.5		
Silver nitrate	19.2 ^a			
Silver sulphate	12.2 ^a			
Zinc oxide	51.4			
Copper carbonate		52.2		
Cuprocide	58.2	60.3	44.4	45.6
Iron sulphate	10.4			67.8
Sulphur (micro)		22.3		
Semesan				39.2
Boric acid	11.4			
Fermate		72.3	65.4	59.0
Phenothiazine, micronized				43.8
Spergon	55.2		52.6	
T.M.D.S.				60.6
No treatment	13.4	30.3	27.4	49.0
Least Mean Sign. Diff. 5 per cent	10.79	9.76	7.27	8.90

^a Diluted, one part silver salt to nine parts bentonite.

^b Diluted, one part silver salt to nine parts infusorial earth.

^c Finely powdered $\text{FeSO}_4 \cdot 1.5\text{H}_2\text{O}$.

trials. In these tests the seeds were treated at the maximum load of material the seeds would retain, the presumably more toxic silver salts being diluted before application. Final counts, presented in table 1, were made a week or less after planting and present no data on the control of post-emergence damping-off. In fact, it is probable that additional emergence would have been recorded had later readings been made on these tests. Failure to emerge under these conditions may have been due to chemical injury of the seedlings, to pre-emergence damping-off, or to a combination of both factors.

As a result of these trials, silver carbonate, Fermate, Spergon, and T.M.D.S. (tetramethyl thiuramdisulphide, 80 per cent active grade, processed as "Jap Beetle Spray") were selected for further tests on a number of different crops.

TABLE 2.—Percentage seedling emergence in preliminary trials on seed treatment

	Emergence							
	In greenhouse				In field			
	Cab- bage	Carrot	Lettuce		Musk- melon	Tomato	Sweet corn	Peas
			1	2				
Date planted	June 19	July 11	June 19	July 9	June 5	June 30	June 16	June 15
Days after planting	5	11	8	8	10	8	9	10
Soil pH	5.25	5.4	5.25	5.4	5.4	5.4	7.4	7.4
Material: ^a								
Fermate	93.2	51.6	9.0	15.2	70.0	88.8	92.4	89.0
T.M.D.S.	93.6	53.2	32.6	26.0		88.8	94.0	86.2
Spergon	89.6	56.0	35.6	33.0	72.8	89.6	89.2	86.0
Cuprocide	74.2	53.8	29.6	39.0	70.4	88.4	85.4	88.0
Silver carb.	86.0	45.2	26.2	10.6	71.1	79.8 ^b	78.6	67.2 ^c
Not treated	77.6	40.0	50.0	38.0	4.4	76.2	79.4	76.0
Least Mean Sign.								
Diff. 5 per cent	6.95	7.85	12.37	5.60	4.42	4.92	5.76	5.49

^a All treatments at maximum dosage with the exception of sweet corn, where 1 gram of material was applied to 180 grams of seed.

^b Pronounced stunting of seedlings.

^c Severe post-emergence injury to seedlings.

The results of these tests on other vegetable seeds are shown in table 2. Here Fermate, T.M.D.S., Spergon, and Cuprocide were approximately equal in effect on seedling emergence on all crops excepting lettuce, the one striking exception being the reduced stand where Cuprocide was applied to cabbage seed (an effect which has been reported many times). With lettuce the picture was less clear and no benefit was obtained from any treatment. In considering these data it should be emphasized that, except on sweet corn, all materials were applied at the maximum amount which would remain on the seed. With some kinds of seeds an excessively high dosage must have been applied, since later tests showed that lettuce would retain Fermate to the extent of approximately 12 per cent of the seed weight. It is probable

that such excessive rates of treatment were conducive to seedling injury on lettuce.

METHODS

Preliminary trials indicated differences between test materials in their ability to reduce or delay post-emergence damping-off. The following methods were employed to demonstrate such differences, and to achieve some standardization in technique.

Seed Treatment. The seeds to be treated were weighed on an analytical balance (0.1 mg. sensitivity). The test material was added to the seeds which then were shaken vigorously in a closed vial, then shaken lightly over a 40-mesh screen to remove unattached particles of the test material, and reweighed. If the desired dosage had not been reached, more material was added, shaken, and screened as before. If the desired dosage was not approximated in three attempts, a fresh start was made with untreated seeds.

This method proved satisfactory with cabbage, carrot, cucumber, lima bean, pea, and spinach seed. With lettuce it was necessary to expose the seeds for some hours in the balance room to prevent humidity differences from causing variations between successive weighings. With seeds not adjusted to the humidity of the balance room such variations frequently were greater than the desired weight of test material.

The treatment method described was unsatisfactory with tomatoes because the abrasive action of the shaking removed hairs from the seed coat and caused losses in weight; such losses varied irregularly with the amount of shaking. Here, the treatment was accomplished by dilution of the test material with talc. Dosage rates were obtained by differential dilution, applying the full load of material that the seed would retain.

Seed Selection. The spinach seed was screened and only those seeds which passed through an 8-mesh, but not through a 10-mesh, screen were used. The larger seeds thus removed gave a lower rate of germination than did the medium-size seeds. The cabbage seed was screened to remove the smallest seeds. The other kinds of seeds were not sized.

Seed-counting Procedure. The seeds were stored in vials until planted, at which time they were counted and distributed immediately into the row. Counting was facilitated by the use of a 1.5 × 5-inch card folded into a trough. A small quantity of seeds was poured on the card, the required number counted, and then poured from the card directly into the row. This procedure avoided a considerable amount of handling of seeds and minimized the loss of material from the seeds before planting. Distribution of the seeds in the row was corrected by moving seeds with forceps, though little of this was needed if sufficient care was exercised.

Soil. Clay loam at pH 5.4, with sufficient sand added to prevent severe crusting, was used throughout these experiments. Initially it was infested heavily with a freshly isolated culture of *Pythium ultimum*. Before starting each test the required amount of soil was screened and mixed by repeated shoveling to insure uniformity.

Where the possibility of chemical injury to the seed was anticipated, a duplicate planting was made using soil from the same source, but partially sterilized with formaldehyde according to the method of Guterman and Massey (13). This was done in order to differentiate between reduced emergence due to chemical injury and low emergence due to poor protection against pre-emergence damping-off. The method of soil sterilization proved satisfactory in controlling pre-emergence damping-off. A small amount of post-emergence damping-off occurred, but the distribution of this suggested post-planting contamination. These damped-off seedlings did not complicate the emergence data since they were detected and recorded in the daily counts.

Planting. Greater uniformity of covering of the seeds was achieved by pressing the rows to a constant depth ($\frac{5}{8}$ inch for small seeds, $\frac{3}{4}$ inch for cucumber and other large seeds). The row-press consisted of a $\frac{1}{2}$ inch board having rounded edges with sidepieces adjustable to the desired depth. After the seeds had been spaced, the row was filled with finely screened soil of the same source, dispensed from a large funnel.

Watering. The flats were watered heavily by sprinkling twice daily at a rate to maintain a high soil moisture.

Plant Counts. When an appreciable emergence had occurred, daily counts were made of all plants. Those that had damped-off were recorded and removed. This method of counting provided data on the time of development of post-emergence damping-off with different materials. In the earlier tests 100 seeds were used, each replicated to five plots in a randomized block arrangement. Later the seed number per plot was reduced to 50 with some crops, and to 20 with such crops as cucumbers. The total number of seeds used is shown in the discussion of each test.

Statistical Treatment. The least significant difference between treatment means was determined by the method of Analysis of Variance.

SEED TREATMENT OF SPINACH--MATERIALS AND DOSAGE

Following the preliminary trials, it was deemed advisable to test the materials at different dosage rates. The plants were counted daily for the first few days after emergence, later at 2-day intervals when most of the plants had attained such size that they were not likely to damp-off and "disappear" during a 48-hour period.

In two trials of spinach (planted September 3 and October 19, 1942) certain materials and dosages were duplicated; these may be considered in a combined analysis of data drawn from the two trials.

The final results of the September 3 test are shown in table 3. Here the daily counts permitted accumulation of the total number of plants damped-off. This total, when added to the number standing at any date, permits the calculation of the total emergence to that date. The difference between the total emergence of plants and the maximum expected emergence of plants (75 to 85 per cent in later trials with partially sterilized soil) may be attributed to pre-emergence damping-off.

TABLE 3.—*Results of greenhouse test on spinach planted Sept. 3, 1942. Five replications of 100 seeds each*

Material	Dosage (Per cent) ^a	Percentage of plants 16 days after planting		
		Surviving	Damped-off	Emerged
T.M.D.S.	2.76	58.6	7.8	66.4
Do	0.97	62.6	10.6	73.2
Do	0.50	55.0	10.6	65.6
Do	0.32	53.8	13.4	67.2
Do	0.12	47.8	16.0	63.8
Fermate	7.38	48.4	18.2	66.6
Do	0.54	47.2	22.0	69.2
Do	0.31	38.4	25.6	64.0
Do	0.13	41.6	18.2	59.8
Spergon	0.33	13.8	16.4	30.2
Cuprocide	2.00	20.2	36.8	57.0
No treatment		8.2	11.6	19.8
Least Mean Sign. Diff.				
5 per cent		9.25	8.27	7.62
1 per cent		12.38	11.06	10.20

^a Throughout this study, dosage refers to the weight of the treatment material expressed as percentage of seed weight.

In this test T.M.D.S. and Fermate, even at dosages as low as 0.1 per cent, resulted in plant stands at 16 days which were significantly higher than those where Cuprocide and Spergon were used at their recommended rates. Spergon, as used, did not significantly improve the stand over the controls. The effect of treatment on emergence of plants was essentially similar to the effect on survival of plants, an exception being found with Cuprocide which

TABLE 4.—*Results of greenhouse test on spinach planted Oct. 19, 1942. Five replications of 100 seeds each*

Material	Dosage (Per cent)	Percentage of plants					
		16 days after planting			24 days after planting		
		Surviving	Damped-off	Emerged	Surviving	Damped-off	Emerged
T.M.D.S.	2.55	60.0	7.4	67.4	53.4	14.2	67.6
Do	1.22	51.2	16.2	67.4	44.6	23.0	67.6
Do	0.51	44.6	22.2	66.8	38.6	29.0	67.6
Do	0.28	38.2	21.2	59.4	32.2	27.8	60.0
Do	0.09	45.2	17.4	62.6	38.4	24.8	63.2
Thiosan	2.80	61.0	4.2	65.2	54.4	11.0	65.4
Do	1.41	50.6	12.2	62.8	43.2	19.6	62.8
Do	0.52	49.0	20.0	69.0	42.2	26.8	69.0
Fermate	7.68	52.2	17.8	70.0	42.6	28.2	70.8
Do	0.54	34.2	26.4	60.6	28.2	32.8	61.0
Spergon	0.33	8.8	18.6	27.4	6.4	21.0	27.4
Zinc oxide AAZ	0.99	17.4	19.2	36.6	12.8	23.8	36.6
Cuprocide	2.00	37.6	16.8	54.4	28.8	25.6	54.4
No treatment		9.8	10.8	20.6	6.2	14.4	20.6
Least Mean Sign. Diff.							
5 per cent		14.07	9.69	9.10	13.11	9.93	9.11
1 per cent		18.76	12.92	12.14	17.47	13.24	12.14

provided fair protection against pre-emergence damping-off, but permitted excessive post-emergence damping-off. Spergon provided little protection against damping-off at any stage.

In the October experiment (Table 4) Thiosan (a wettable preparation containing 50 per cent tetramethyl thiuramdisulphide) was tested and found to be comparable at equal rates of active material to the 80 per cent

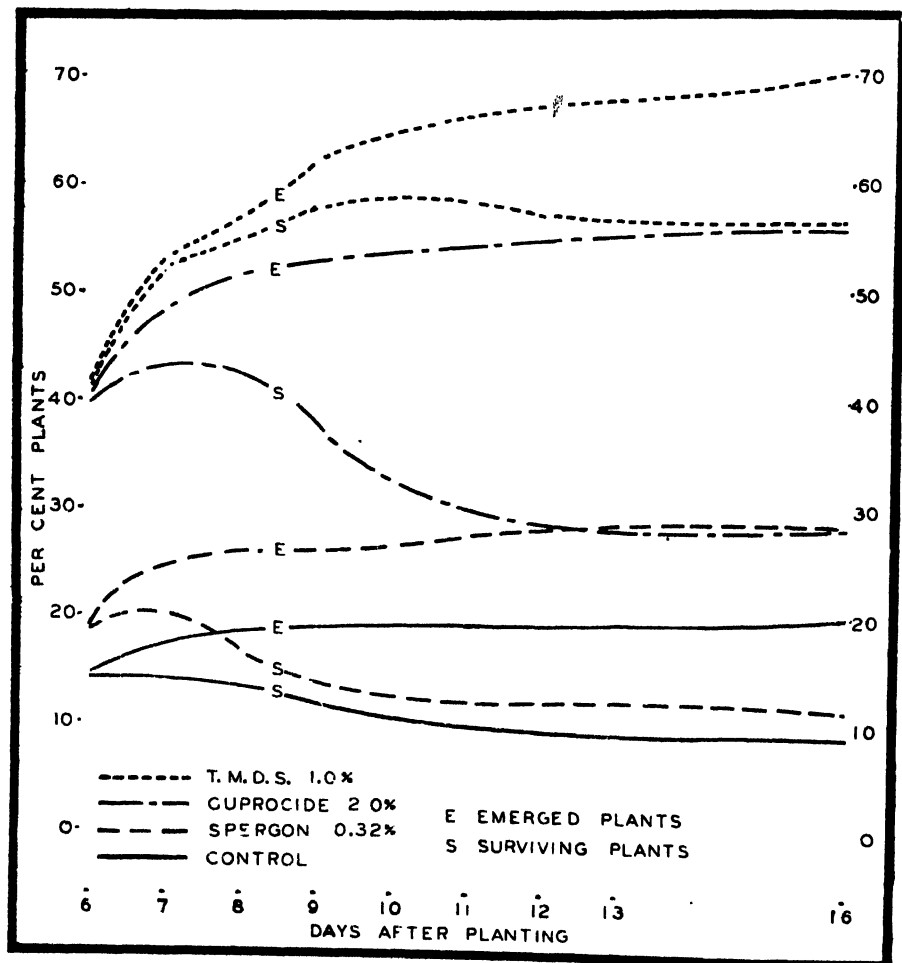


FIG. 1 EMERGENCE AND SURVIVAL OF SPINACH SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT, - COMBINED DATA - AVG. 1000 SEEDS

form (T.M.D.S.) previously used. In this test Cuprocide was more effective than in the September test (Cuprocide plots in both tests were planted from a lot of seeds treated in late August); hence, only the highest dosages of T.M.D.S. and Fermate gave significantly higher stands than Cuprocide. Spergon and zinc oxide failed to protect spinach adequately in this test.

The data from treatments common to both tests were combined, the

homogeneity test described by Hayes and Immer (15) showing that the variances of these two tests are homogeneous. These combined data show that, at similar dosages, T.M.D.S. resulted in significantly more surviving plants than did treatment with Fermate, though the total emergence was approximately equal. Reduction of the dosage with both these materials resulted in reduced control of both pre-emergence and post-emergence damping-off. Cuprocid was significantly poorer than T.M.D.S. and Fermate for plant survival, for post-emergence control, and, with the exception of the lowest

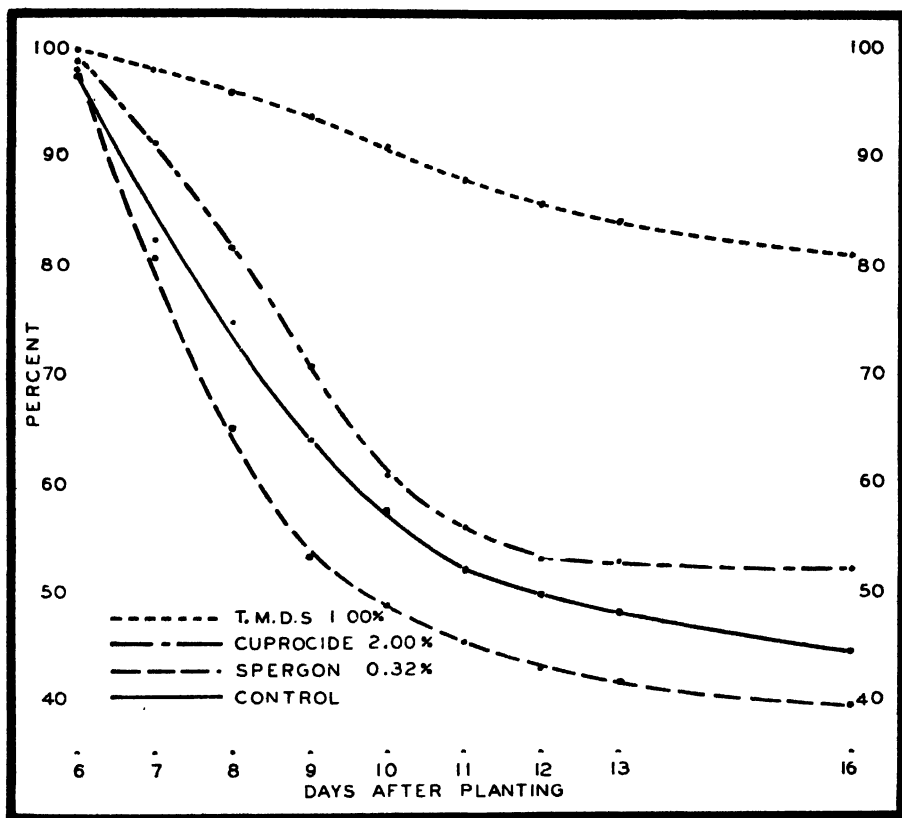


FIG. 2.—PER CENT SURVIVAL OF EMERGED SPINACH SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT

dosages of these materials, for pre-emergence control of damping-off. Spergon was barely significant over no treatment in pre-emergence control and was very ineffective in the control of post-emergence damping-off.

The daily progress of seedling emergence and damping-off, as shown by plant survival, with the combined data of a few selected treatments, have been charted in figure 1. This shows the effect of T.M.D.S. in the protection of the late emerging seedlings, also the comparatively low mortality of the emerged seedlings under conditions highly conducive to damping-off. Noteworthy is the failure of Cuprocid to control post-emergence damping-off as

shown by the early and heavy loss of plants. The ineffectiveness of Spergon is shown by the similarity in slope of the curves for this material and the controls.

To further examine the effectiveness of protection against post-emergence damping-off afforded by different treatment materials, the percentage of survival of those plants which emerged is charted in figure 2. This chart shows that post-emergence damping-off with Cuprocide, Spergon, and with no treatment is early and rapid and follows a very similar pattern, as con-

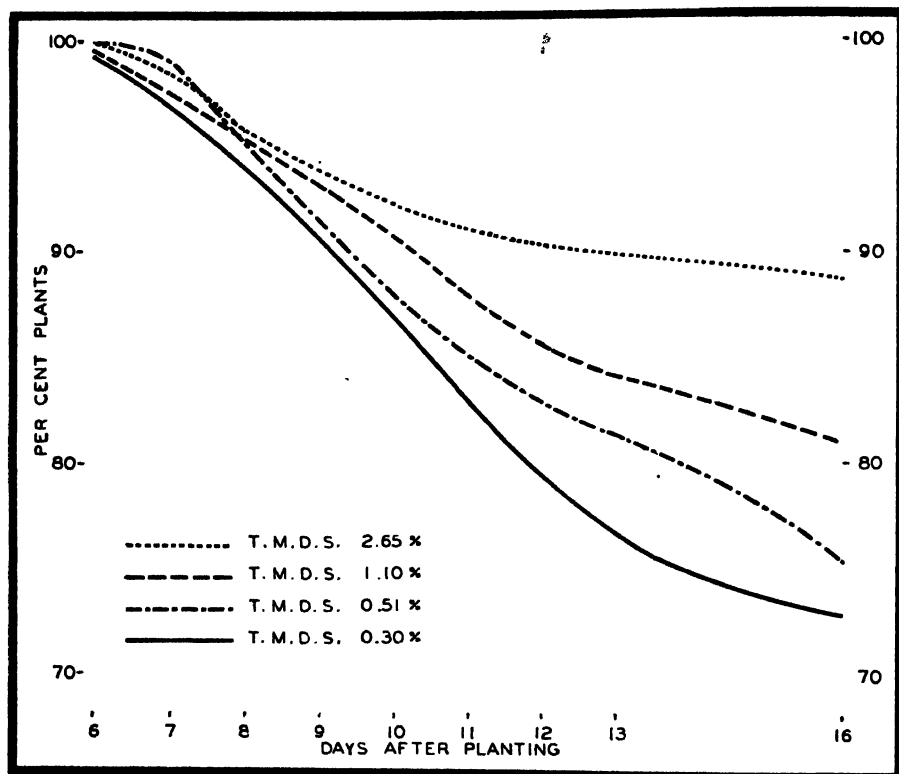


FIG. 3.— PER CENT SURVIVAL OF EMERGED SPINACH SEEDLINGS AS A FUNCTION OF TIME AND DOSAGE OF TETRAMETHYL THIURAMDISULPHIDE

trasted with the delayed pattern of post-emergence damping-off with T.M.D.S. at one per cent. It should be noted that the data in this figure were obtained by the formula $\text{number surviving} \times 100 / \text{number which had emerged}$. Thus, 100 surviving of a total of 200 emerged plants would give the same survival percentage as 250 surviving from a total emergence of 500 plants, even though the latter represents a much better stand of plants from the 1000 seeds planted.

Data from these two experiments are available on the effect of different dosages of tetramethyl thiuramdisulphide on the control of post-emergence damping-off. The percentage survival of emerged plants, as charted in fig-

ure 3, shows that the control of post-emergence damping-off increased as the treatment rate was increased.

TETRACHLORORESORCINOL AND COPPER TRICHLORPHENATE AS SEED
PROTECTANTS ON SPINACH

Tetrachlororesorcinol and C-119 (a preparation containing 20 per cent copper trichlorophenate) at two dosages each were compared with Thiosan at 1.5 per cent in one test, table 5 and figure 4.

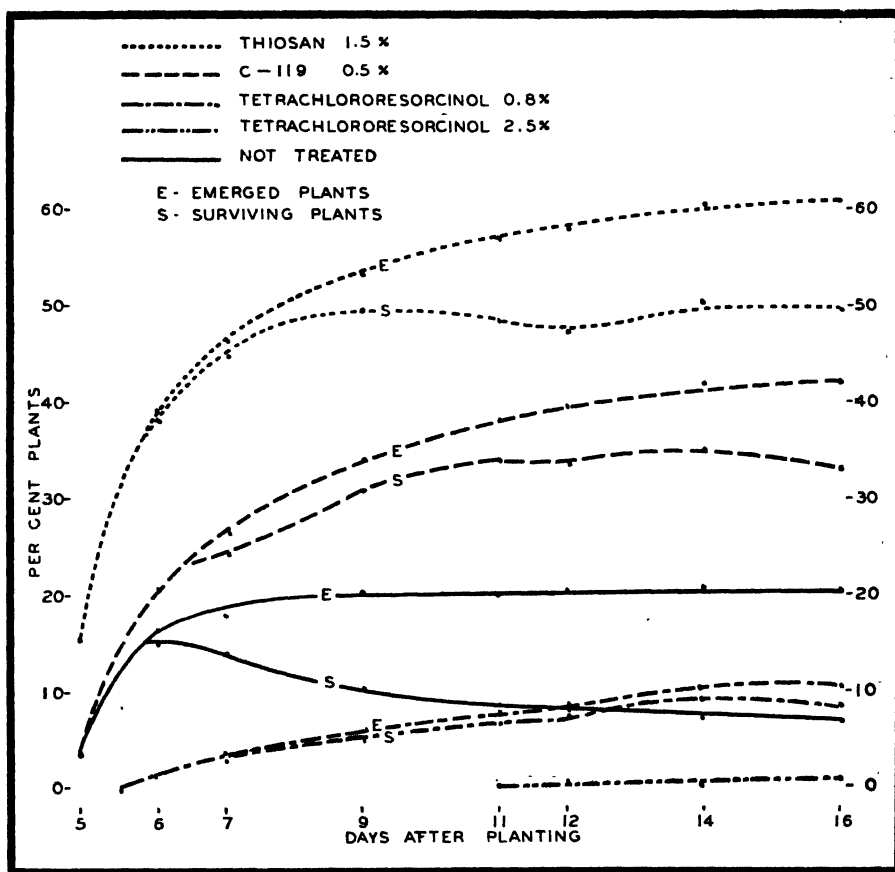


FIG. 4.—EMERGENCE AND SURVIVAL OF SPINACH SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT WITH TETRACHLORORESORCINOL AND COPPER TRICHLORPHENATE

These data show that tetrachlororesorcinol at 2.5 per cent dosage permitted the emergence of but three plants from 500 seeds, but that a 12.2 per cent emergence occurred with a dosage of 0.8 per cent. While this experiment was not duplicated in partially sterilized soil, the data show clearly that the low emergence was due to injury and not to lack of protection. This injury was further demonstrated by the delayed emergence (Fig. 4). At the lower dosage of 0.8 per cent, 23 per cent of those plants which had

TABLE 5.—*Copper trichlorophenate and tetrachlororesorcinol as seed protectants on spinach planted Oct. 23, 1942. Five replications of 100 seeds each*

Material	Dosage (Per cent)	Percentage of plants 18 days after planting		
		Surviving	Damped-off	Emerged
Thiosan	1.5	48.4	12.6	61.0
C-119	1.5	12.0	0.8	12.8
Do	0.55	32.8	9.8	42.6
Tetrachlororesorcinol	2.5	0.6	0.0	0.6
Do	0.8	9.4	2.8	12.2
No treatment ..		9.4	13.4	21.8

emerged had damped-off within 18 days after planting. This result suggests that protection against damping-off would not be satisfactory at dosages sufficiently low to be non-injurious.

C-119 at 1.5 per cent also proved to be excessively injurious, giving both delayed and low emergence. However, only 6.3 per cent of those plants which emerged damped-off within 18 days, thus showing a high order of protection against post-emergence damping-off. At the lower dosage of 0.5 per cent, while the emergence was considerably lower than with Thiosan, post-emergence protection was excellent. Further trials of these materials were not made, inasmuch as they appeared unsafe on spinach.

SILVER SALTS AS SEED PROTECTANTS ON SPINACH

The promising results obtained with silver carbonate in the preliminary trials prompted further testing of this material, together with silver chro-

TABLE 6.—*Silver salts compared with other seed protectants on spinach, planted Dec. 28, 1942. Five replications of 50 seeds each*

Material	Dosage (Per cent)	Percentage of plants 16 days after planting			
		Infested soil		Partially sterilized soil	
		Surviving	Damped-off	Emerged	Emerged
Silver carbonate ..	3.82	10.4	38.8	49.2	76.0
Do ..	0.99	9.6	44.8	54.4	78.3
Do ..	0.31	9.6	26.8	36.4	79.2
Silver chromate ..	4.04	52.0	26.8	78.7	76.0
Do ..	0.82	30.8	39.2	70.0	81.6
Silver oxide ..	2.12	10.0	55.2	65.2	73.6
Do ..	0.40	13.6	37.2	50.8	76.0
Sil. orthophosphate ..	2.76	18.0	41.2	59.2	77.2
Do ..	0.27	4.8	22.4	27.2	73.2
T.M.D.S.	0.99	55.2	25.2	80.4	88.0
Thiosan ..	1.48	58.4	22.4	80.8	82.4
Cuprocide ..	2.00	26.8	37.6	64.4	82.0
Iron sulfate $1.5\text{H}_2\text{O}$..	2.20	27.4	32.4	58.8	78.4
No treatment ..		8.4	13.2	21.6	76.4
Least Mean Sign. Diff.					
5 per cent ..		15.9	14.2	14.1	10.1
1 per cent ..		21.1	18.9	18.8	

mate, silver oxide, and silver orthophosphate. These were pulverized in an agate mortar to pass through a 100-mesh screen to insure small particle size. By such means it was possible to apply dosages sufficient to detect any tendency toward seedling injury. To explore further any tendency to cause injury, this experiment was duplicated in soil from the lot discussed under "Methods." However, in this duplicate test the soil was partially sterilized by the formaldehyde treatment method of Guterman and Massey (13).

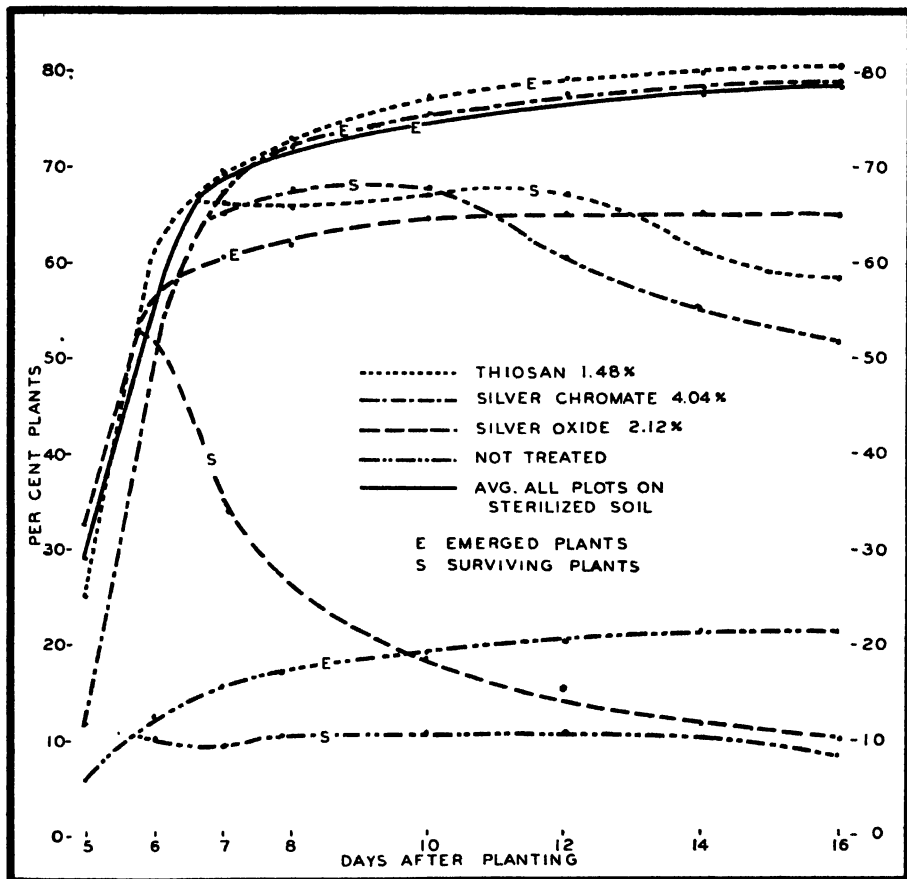


FIG. 5.—EMERGENCE AND SURVIVAL OF SPINACH SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT — 250 SEEDS PER TREATMENT.

Table 6 shows that none of these materials significantly reduced the plant stand at 16 days when planted in partially sterilized soil; however, data taken during the period of most rapid emergence suggest that high dosages of silver chromate delayed emergence slightly. Silver chromate at 4.0 per cent dosage gave sufficient protection to result in a final stand comparable to that obtained with T.M.D.S.; at 0.8 per cent it was much less effective, being nonsignificantly better than Cuprocide in any respect. Silver carbonate, silver orthophosphate, and silver oxide were ineffective in protecting the

emerged plants against post-emergence damping-off though each, with the exception of silver orthophosphate at 0.2 per cent dosage, offered a considerable degree of protection through the pre-emergence period. The iron sulphate tested was surprisingly effective in this test, being as effective as Cupro-cide. A further comparison between T.M.D.S. and Thiosan at approximately equal dosages of tetramethyl thiuramdisulphide showed these materials to be of equal effectiveness and to be significantly better than any other materials in this test, with the exception of silver chromate at 4.0 per cent dosage. With these three materials, the number of plants which emerged in heavily infested soil approached closely the number which emerged in

TABLE 7.—Seed protectants on *Big Boston lettuce* grown in the greenhouse

Material	Dosage (Per cent)	Five replications of 100 seeds, planted Sept. 26, 1942 Percentage of plants at 15 days			Four replications of 50 seeds, planted Nov. 21, 1942 Percentage of plants at 16 days		
		Sur- viving	Damped off	Emerged	Sur- viving	Damped- off	Emerged
T.M.D.S.	4.18	69.2	1.8	71.0	60.5	4.5	65.0
Do	0.99	80.2	1.2	81.4	70.5	6.5	77.0
Do	0.50	82.6	2.4	85.0	59.5	16.0	75.5
Do	0.29	79.0	2.8	81.8	50.5	18.5	69.0
Do	0.15	78.0	3.2	81.2	64.5	8.0	72.5
Thiosan	3.25				56.5	4.0	60.5
Do	1.65	81.0	1.0	82.0	58.0	10.0	68.0
Do	0.75				49.0	16.0	65.0
Fernate	11.96	75.2	0.8	76.0	57.5	4.5	62.0
Do	1.00	84.6	2.2	86.8	61.0	14.5	75.5
Do	0.56	77.0	3.6	80.6	55.5	12.0	67.5
Do	0.38	73.8	4.2	78.0	60.5	13.0	73.5
Do	0.15	66.8	5.6	70.4	46.5	12.0	58.5
Spargon	0.28	76.0	2.4	78.4			
Cupro-cide	1.82	93.0	2.2	95.2	82.5	3.5	86.0
No treatment		35.4	4.0	39.4	26.5	5.5	32.0
Least Mean Sign. Diff.							
5 per cent		7.1	Not	6.0	16.1	8.6	13.3
1 per cent		9.4	sign.	8.0	21.5	11.5	17.7

partially sterilized soil, thus showing their very great effectiveness in preventing pre-emergence damping-off.

The transient nature of the protection offered by most of the silver salts is well illustrated by silver oxide at 2.1 per cent dosage, as plotted in figure 5. With this material a stand of 52.4 per cent was reached on the sixth day, but this stand was reduced by post-emergence damping-off to 25.2 per cent by the eighth day and to 10 per cent by the sixteenth day. That this material afforded a considerable amount of pre-emergence protection is shown by the total emergence of 65.2 per cent. Silver chromate, on the other hand, when used at 4.0 per cent dosage, gave a high degree of protection throughout this experiment, though causing a slight lag in emergence on the fifth and sixth days. The excellent protection against pre-emergence damping-off provided by silver chromate at 4.0 per cent and by T.M.D.S. and Thiosan

is evidenced by the fact that emergence with these materials in infested soil was equal to the average emergence with all treatments on partially sterilized soil (shown as the solid line in figure 5).

SEED PROTECTANTS ON LETTUCE

Two trials were conducted on Big Boston lettuce, the data from which are presented in table 7. In one of these 100 seeds were planted in each of

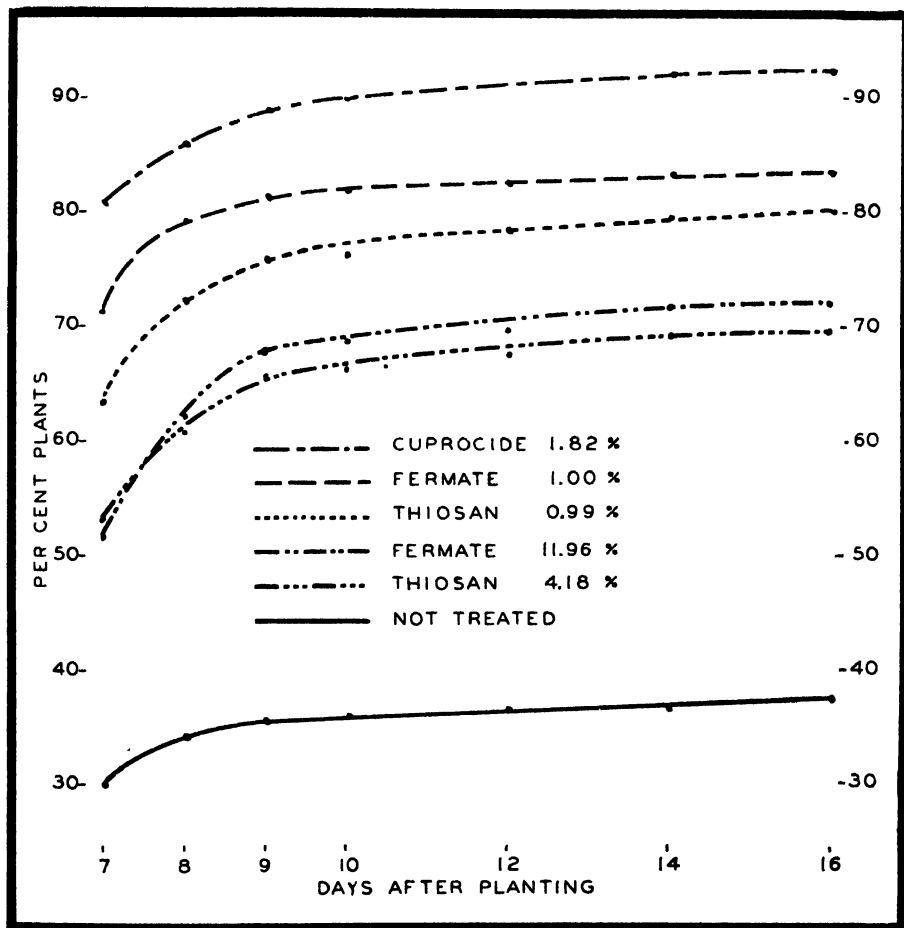


FIG. 6-EMERGENCE OF LETTUCE SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT - 700 SEEDS PER TREATMENT.

five replications; in the other, 50 seeds were planted in each of four replications.

In these experiments all materials tested gave very significant increases in stand over the untreated controls. Cuprocide, at 1.82 per cent dosage, gave excellent results in both trials, being significantly better than most of the other treatments. With T.M.D.S. and with Fermate, the optimum dosage

appeared to be near 0.5 to 1.0 per cent. The tendency towards greater incidence of post-emergence damping-off at the lower dosages of these materials suggests that the lower emergence here probably was due to failure to control adequately pre-emergence damping-off. The reduced stands with the highest dosage of these materials is believed to have been due to injury to the seeds or seedlings. The plants in the highest dosage plots were dwarfed slightly throughout the experiments, supporting the probability that injury was a factor in this case.

The data on plant emergence from the combined tests are presented in figure 6 and serve to illustrate the greater benefits obtained from the use of Cuprocid. This figure also illustrates the lower stands when more than the optimum dosage was applied with both T.M.D.S. and with Fermate.

SEED PROTECTANTS ON CUCUMBER

Table 8 presents the results obtained from the use of various protectants on cucumber seed. All treatments tested gave highly significant increases

TABLE 8.—*Effect of seed protectants on cucumber planted Feb. 24, 1943. Six replications of 40 seeds each*

Material	Dosage (Per cent)	Percentage of plants 17 days after planting		
		Surviving	Damped-off	Emerged
Thiosan	0.25	79.2	7.1	86.3
Do	0.12	77.1	10.8	87.9
Do	0.05	70.0	13.8	83.8
Fermate	0.20	74.2	12.5	86.7
Do	0.10	78.8	7.5	86.3
Do	0.05	76.3	10.0	86.3
Spergon	0.23	60.4	19.2	79.6
Do	0.13	62.5	20.4	82.9
FeSO ₄ · 1.5H ₂ O	0.63	51.7	18.8	70.4
Semesan	0.22	69.6	17.9	87.5
Cuprocid	0.31	63.8	19.6	83.3
No treatment		5.8	12.9	18.8
Least Mean Sign. Diff.				
5 per cent		16.1	Not	9.1
1 per cent		21.4	sign.	12.1

over the untreated control in emergence and in the final stand at 17 days after planting. Among the treatments only the iron sulphate was significantly less effective than any other material at the 1 per cent level. An apparent trend toward lower amounts of post-emergence damping-off with Thiosan and with Fermate, particularly at the higher dosages, proved to be non-significant upon statistical examination. The daily progress of emergence and damping-off for a few materials is charted in figure 7. Here the marked increase in stand from the use of any of these treatments is apparent by reference to the untreated control.

CABBAGE SEED TREATMENT

Since Cuprocid repeatedly has been shown to be injurious to members of the Brassicaceae, a test of some of the newer materials was conducted on

cabbage. The results of a greenhouse test are shown in table 9 and in figure 8.

In this test, treatment with Thiosan, Fermate, and zinc oxide yielded approximately equal emergence of plants. All dosages of Thiosan and

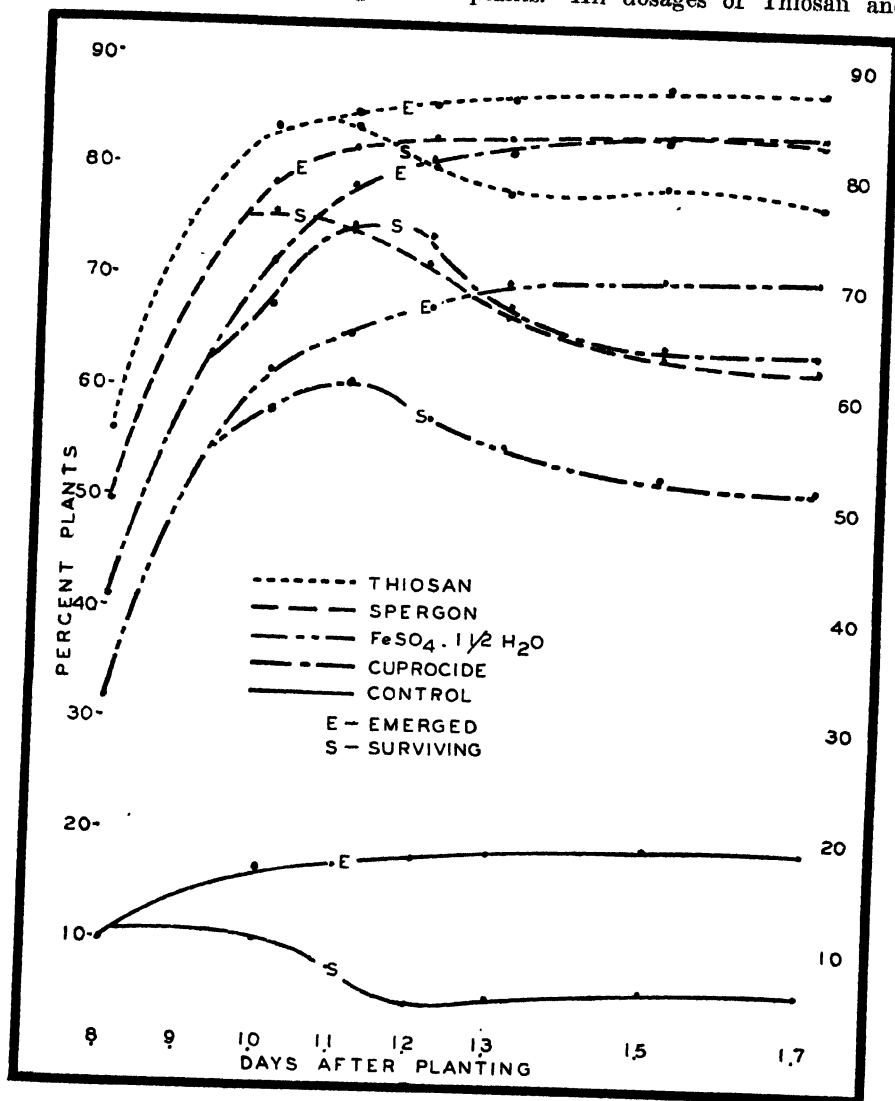


FIG. 7.- EMERGENCE AND SURVIVAL OF CUCUMBER SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT — 240 SEEDS PER TREATMENT

T.M.D.S. resulted in an average emergence of 79.3 per cent, while all dosages of Fermate averaged 79.2 per cent. In this experiment post-emergence damping-off was so slight that no conclusions may be drawn on this phase of protection.

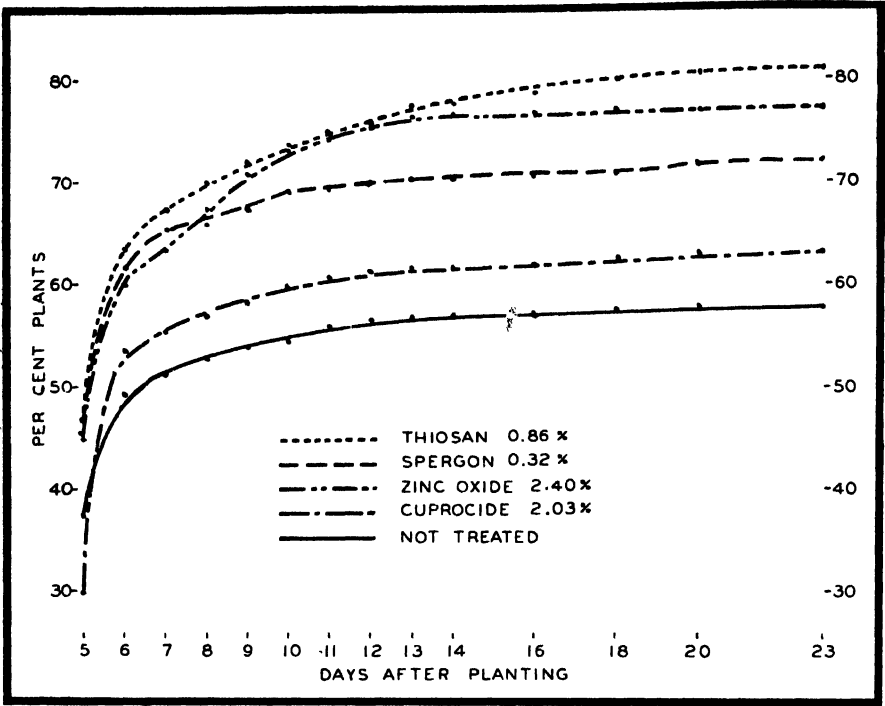


FIG 8- EMERGENCE OF CABBAGE SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT — 300 SEEDS PER TREATMENT.

TABLE 9.—*Effect of seed protectants on cabbage planted Nov. 17, 1942. Six replications of 50 seeds each*

Materials	Dosage (Per cent)	Percentage of plants 23 days after planting		
		Surviving	Damped-off	Emerged
T.M.D.S.	0.56	83.3	1.7	85.0
Thiosan	3.11	77.3	1.0	78.3
Do	1.48	77.0	2.0	79.0
Do	0.86	78.3	2.3	80.7
Do	0.45	70.0	5.3	75.3
Do	0.23	73.3	4.3	77.7
Formate	3.21	84.3	0.3	84.7
Do	0.95	79.3	1.0	80.3
Do	0.52	79.0	3.3	82.7
Do	0.27	72.7	2.3	75.0
Do	0.15	72.7	1.0	73.7
Spergon	0.32	71.0	1.0	72.0
Zinc oxide	2.40	77.0	0.0	77.0
Semesan	0.42	71.0	1.7	72.7
Cuprocid	2.03	59.0	4.0	63.0
No treatment		55.3	2.0	57.3
Least Mean Sign. Diff.				
5 per cent		7.0	Not sign.	6.3
1 per cent		9.3		8.4

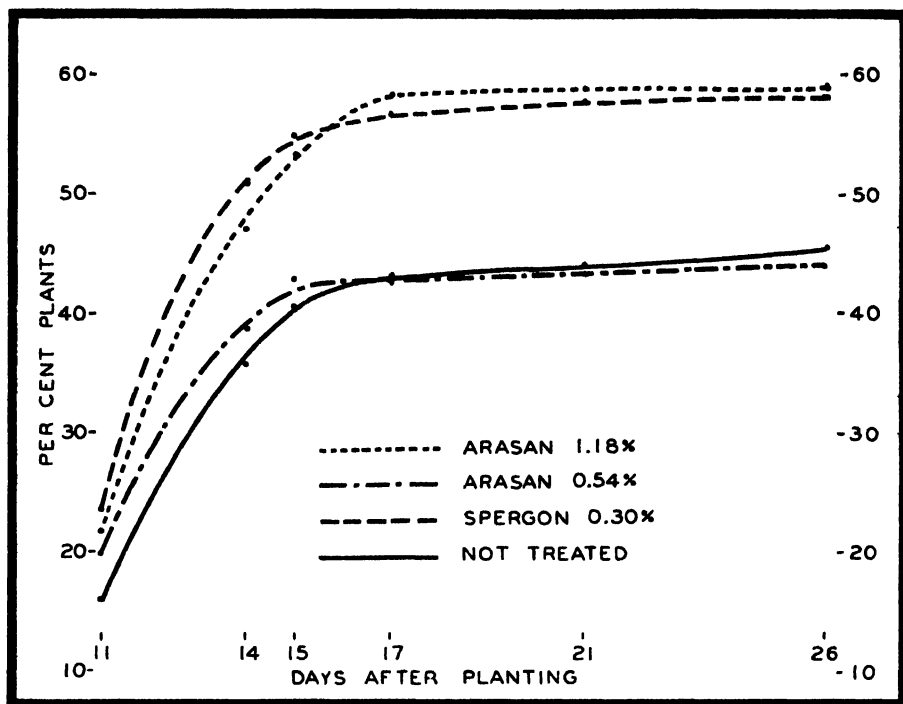


FIG. 9.— EMERGENCE OF CARROT SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT — 250 SEEDS PER TREATMENT

Treatment with Spergon and with Semesan, at recommended dosages, resulted in slightly lower emergence than did treatment with Thiosan, Fermate, and zinc oxide. The daily progress of emergence with Spergon, figure 8, was very similar to that of Semesan (not shown).

TABLE 10.—*Effect of seed protectants on carrot planted Apr. 6, 1943. Five replications of 50 seeds each*

Materials	Dosage (Per cent)	Percentage of plants 26 days after planting		
		Surviving	Damped-off	Emerged
Arasan	2.24	58.0	0.8	58.8
Do	1.18	58.0	1.2	59.2
Do	0.54	42.0	2.0	44.0
Do	0.26	42.4	1.6	44.0
Fermate	4.75	55.6	0.0	55.6
Do	1.10	57.2	1.2	58.4
Do	0.52	47.6	1.6	49.2
Do	0.27	52.0	1.2	53.2
Spergon	0.30	55.6	2.4	58.0
Semesan	0.42	56.0	4.0	60.0
Cuproide	2.28	52.8	1.6	54.4
No treatment		40.8	2.4	45.6
Least Mean Sign. Diff.				
5 per cent		7.76	Not	7.6
1 per cent		10.38	sign.	10.2

Cuprocide, in this test, resulted in emergence significantly lower than that in any other treatment series and the emergence was not significantly higher than that with no treatment.

CARROT SEED TREATMENT

A single experiment was conducted on the treatment of carrot seed. For this trial Arasan was available and, since this appeared to be the form in which tetramethyl thiuramdisulphide would be marketed as a seed treatment material, it was substituted for T.M.D.S. and Thiosan.

In this experiment, only Arasan at 0.5 per cent or lower dosage failed to give appreciable increases in plant emergence and survival (Table 10 and Fig. 9). Arasan and Fermate at dosages of 1.1 per cent or higher, Spergon at 0.3 per cent, Semesan at 0.42 per cent, and Cuprocide at 2.3 per cent all offered such protection that both the emergence and the survival of plants were significantly better than those of the controls.

The incidence of post-emergence damping-off was low in this experiment and no significant differences in its control were obtained.

TOMATO SEED TREATMENT

Arasan, Thiosan, Fermate, Spergon, Cuprocide, and talc were tested on tomato seed in a single trial. Here dosage rates were achieved by dilution of the treatment material. The data from this test are not presented since an excellent stand of plants was obtained regardless of treatment, the controls being nonsignificantly poorer than any of the protective treatments.

DISCUSSION

In these studies the practice of making frequent counts of healthy and of damped-off seedlings has permitted graphic portrayal of the response to the use of various seed protectants. Spinach, being highly susceptible to damping-off both before and after emergence from the soil, was found to be especially suited to the testing of new materials.

Under conditions of temperature and moisture favorable to attack by *Pythium ultimum*, Arasan and the other tetramethyl thiuramdisulphide mixtures proved to be very effective protectants against pre-emergence damping-off. They were more effective than the already widely used Cuprocide, Spergon, and, where tested, Semesan. However, the most striking effect of Arasan and of related materials has been found in the high degree of protection they have offered against post-emergence damping-off. This continued protective value promises great effectiveness where seed germination is long-delayed by unfavorable weather conditions. The effect of Arasan in protecting against such unfavorable weather conditions is directly proportional to the dosage applied and it, therefore, is important that seeds not only be treated but that adequate dosages be used.

Fermate approached Arasan closely in its effectiveness against both pre-emergence and post-emergence damping-off. However, the black color of

this material, as compared with the pink color of Arasan, offers a disadvantage where seeds are not to be machine planted. In hand seeding it is very difficult to determine the spacing with Fermate-treated seeds due to their inconspicuous color.

Cuprocide was reasonably effective in preventing pre-emergence damping-off, but this protection lasted for a very few days. After emergence, plants from Cuprocide-treated seeds frequently were as subject to post-emergence damping-off as were seedlings from untreated seeds.

Sperguson, when used on spinach at dosages recommended by the manufacturer, was inadequate under the conditions of these tests. Its low effectiveness on spinach is in agreement with the findings of other workers.

The use of duplicate plantings in partially sterilized soil offers a method of differentiating between low emergence due to chemical injury to the seedling and low emergence due to poor protection against pre-emergence damping-off.

SUMMARY

1. A study was made of the value of numerous chemicals in combating damping-off when dusted on vegetable seeds. Particular attention was given to refinements in technique so that the relative values of the materials and dosages could be compared more precisely. Plant counts at frequent intervals permitted study of the influence of the protectant material on the control of damping-off at different stages in the development of the seedling.

2. Arasan and other mixtures of tetramethyl thiuramdisulphide were the most effective of the seed protectants tested, except with lettuce. Their protective influence continued into the seedling stage to a greater extent than occurred with the previously used seed protectants.

3. The protective value of Arasan and of related materials was in direct proportion to the dosage applied.

4. Fermate approached Arasan in effectiveness as a seed protectant.

5. The protective effect of Cuprocide was dissipated within a few days under the conditions of these tests.

6. Silver chromate at very high dosages offered good protection; silver oxide was protective for a very few days.

7. Sperguson was low in protective value.

8. Seed treatment with Arasan was successful with spinach, cucumber, carrot, cabbage, and tomato. On lettuce both Arasan and Fermate were less beneficial than Cuprocide, the data indicating that Arasan and Fermate were injurious to lettuce.

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A STUDY OF THE OCCURRENCE OF PHOMOPSIS AND OF DIPLODIA ROTS IN FLORIDA ORANGES UNDER VARIOUS CONDITIONS AND TREATMENTS

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(Accepted for publication June 3, 1946)

Although stem-end rot of oranges is usually considered as one disease, it is well known that two causal organisms, *Phomopsis citri* Faw. (*Diaporthe citri* (Faw.) Wolf) and *Diplodia natalensis* Evans (*Physalospora rhodina* (B. & C.) Cke.), are involved. Either organism alone is capable of producing the characteristic rot and because of the similarity of symptoms it is nearly impossible to tell by macroscopic examination which organism is causing stem-end rot in an individual fruit.

To gain insight into the action of each of these fungi, observations were taken and isolations made at weekly intervals from all the fruit suspected of having rot among 850 separate samples of 50 fruits each. These samples were taken from approximately 125 different tests which were set up to study various phases in stem-end-rot control. These tests took into consideration such factors as appear in (1) the groves, (2) picking, (3) packinghouse operations, and (4) storage. Most of the investigations dealt with here are related to the third group. The samples in the various similar tests were so arranged that the data obtained could be treated statistically by the analysis of variance method. The 5 per cent level of probability was chosen as the criterion of significant differences.

EFFECTS OF GASSING, DEBUTTONING BY VARIOUS METHODS, AND BORAX TREATMENTS

In an experiment, consisting of 16 tests obtained from 4 replications of each of the varieties, Hamlin, Parson Brown, Pineapple, and Valencia, throughout the picking season, reported by Hopkins, Loucks, and Stearns,³ a study was made of the effect of various treatments, applied both separately and in combination, on stem-end rot. The single treatments were: gassing with ethylene, for 48 hours at 85° F. and 90 per cent relative humidity, at a concentration comparable to that used in commercial gassing chambers for degreening oranges; removing buttons after 48 hr. gassing; soaking in a 5 per cent solution of borax for 5 min. at 110° F. and not rinsing off except where especially noted. Borax was applied before and/or after gassing. Only those combinations that appeared likely to be effective were tried. *Phomopsis* and *Diplodia* were isolated from decayed specimens of the 50 fruits used for each sample: the percentages of each fungus that

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³ Hopkins, E. F., K. W. Loucks, and C. R. Stearns, Jr. A study of certain methods for the control of stem-end rot and blue mold in oranges. Proc. Fla. State Hort. Soc. 57: 87-98. 1944.

developed within three weeks after treatment are indicated in figures 1 and 2, respectively.

The least difference necessary for significance among the results for *Phomopsis* is 5.2 per cent, and for *Diplodia* 8.6 per cent. In the figures all treatments designated by the same letter are of equal importance so far as the development of the fungi is concerned. For instance, the difference between the average percentage of *Phomopsis* present in 3 weeks from Treatment 11 was not significantly different from that with Treatment 1, but it was significantly different from Treatment 12. Likewise, Treatment 12 was not significantly different from Treatment 8 but was from Treatments 10 and 4. As there was a possibility that longer storage might change the picture, the data for 5 weeks were similarly analyzed, but the results showed that the general trend was still the same as at 3 weeks.

Because 3 weeks is considered to be the nearest representation of the average time which elapses from picking a commercial fruit until it is consumed, the results after 3 weeks are the ones given the most consideration and reported in this paper.

Debuttoning effectively controlled *Phomopsis* (Fig. 1). If the buttons were not removed, borax, alone or used in the various combinations, was no better than the ungassed check (Treatment 8). When used with gassed fruit, either before, or before and after, the ethylene treatment, borax reduced the amount of *Phomopsis*. With only the gas treatment, more *Phomopsis* developed than in the checks. When the buttons were removed from borax-treated fruit, the effect of gassing was overcome. Borax applied before gassing fruit with buttons left on was not better than borax applied after gassing for controlling *Phomopsis*.

Debuttoning also was very effective in reducing *Diplodia* (Fig. 2). Borax applied before, or before and after, gassing was effective in reducing *Diplodia* if the buttons were left on. If the buttons were removed the effect of borax, if any, was masked by the good control attendant upon button removal even though the fruit was gassed. Gassing fruit or leaving the buttons on increased the amount of *Diplodia*.

Another experiment with 4 replications from the varieties Valencia and Pineapple was set up to test the various methods of partially or wholly debuttoning oranges for stem-end-rot control. These were also combined with the borax treatment before and after gassing, and the borax treatments were either left on or washed off. Seventeen predetermined treatment combinations, as shown in figures 3 and 4, were used. The various button treatments were as follows:

- (a) Button left intact by clipping the fruit.
- (b) Calyx removed by pulling fruit or by a drill-press reamer.
- (c) Button completely removed by hand after gassing fruit for 48 hours.

Other treatments were the same as in the previous experiment except that with some of the combinations with borax treatment the borax was washed off after the fruit had soaked for 5 minutes.

The data for the 3-week periods were used to obtain the information presented in figure 3 where the various treatments are arranged in their order of rank in controlling *Phomopsis*. The least difference necessary for significance between treatments at the 5 per cent level is 5.9 per cent. Treatments that do not show this difference are designated by assigning to them the same letter. The striking result is that no matter how the calyx is removed, whether by pulling or by removing the entire button, or by reaming, *Phomopsis* was reduced very significantly regardless of what other treatment or treatments were combined with the debutting. *Phomopsis* was not increased by gassing. Pulled fruit with the calyx left on was not different from clipped fruit (calyx on). Among the various treatments in which borax was used there was not sufficient difference to determine the effect of borax, and nothing was deducible concerning borax left on as against borax washed off.

In figure 4 is presented the same information about *Diplodia*. Here the corresponding least difference between the averages of the percentage of *Diplodia* necessary for significance is 15.4 per cent. The lack of differences is designated by letters in the same manner as in figure 3.

Most striking is the fact that fruit that lost its calyx when pulled from the tree was very susceptible to *Diplodia* when subsequently gassed (Fig. 4). Ethylene applied to fruit that had its calyx intact also was very active in increasing *Diplodia*. This effect of gassing upon *Diplodia* was not overcome by removing the calyx by pulling the fruit, even if borax was applied after the gassing and left on. It was successfully counteracted only by removing buttons or by removing the buttons and then treating the fruit with borax (even though it was washed off) after gassing. Calyx removal by any of the methods used had no effect on *Diplodia*.

No matter how the fruit was otherwise treated, gassing did not significantly increase *Phomopsis* but with few exceptions did very substantially increase *Diplodia*. When the fruit was debudded after gassing, but not otherwise treated, the effect of gassing was overcome. Some of these same results have been reported by Brooks,⁴ Voorhees,⁵ and others.

CORRELATION BETWEEN OCCURRENCE OF PHOMOPSIS AND DIPLODIA

From 170 samples of 50 fruit each, which were picked under similar conditions and used as checks for that many separate experiments, data were obtained concerning the development of *Phomopsis* and of *Diplodia* to determine their interrelationship. Storage conditions were not comparable for all samples, but aside from that the fruit was all treated alike. The fruit was picked by clipping, randomized into samples, and immediately placed in storage. The varieties Hamlin, Parson Brown, Pineapple, and Valencia were represented.

⁴ Brooks, Charles. Stem-end rot of oranges and factors affecting its control. Jour. Agr. Res. [U. S.] 68: 363-381. 1944.

⁵ Voorhees, R. K. Investigations of melanose and stem-end rot of citrus fruit. Ann. Rept. Fla. Agr. Exp. Sta. p. 188-191. 1944.

TABLE 1.—Regression and correlation coefficients, obtained from the data from 4 varieties of oranges, showing the lack of interrelationship between *Phomopsis* and *Diplodia*

Variety	Regression Coefficient	Correlation Coefficient
Hamlin	0.321	0.212
Parson Brown	0.143	0.143
Pineapple	0.041	0.038
Valencia	0.515	0.347 ^a

^a Only data from the Valencia oranges showed significant correlation between the occurrence of *Phomopsis* and of *Diplodia*.

When the data from all varieties were combined to form a correlation table to test relationship between occurrence of *Phomopsis* and *Diplodia* based on results after 3 weeks in storage, the linear regression line had a positive trend but the points were too scattered for the correlation to be significant.

The linear regression was applied to data from each variety separately and the coefficients of regression and correlation coefficients are shown in table 1. These data indicate that whatever combination of factors produced an increase in one of the causal organisms did not cause a decrease in the other and there is no proof that the converse is true. Apparently there is no interrelationship between the two organisms.

HIGH VERSUS LOW RELATIVE HUMIDITY

An experiment was designed to test the influence of relative humidity in the storage room upon the rate of development of *Phomopsis* and of *Diplodia*. Four samples of each of the varieties, Hamlin, Parson Brown, Pineapple, and Valencia, were obtained on March 17, 1944. One-half of the samples from each variety was placed in each of two storage rooms. The temperatures and relative humidities of the two rooms were controlled and obtained as shown in table 2.

The low humidity room was held at $75 \pm 0^\circ$ F. and a relative humidity of 90 ± 4 per cent.

Isolations of *Phomopsis* and *Diplodia* were obtained from the rotten fruit at weekly intervals (Fig. 5). It was found that the least difference necessary for significance at the 5 per cent level of the 3-week and 5-week periods

TABLE 2.—Temperature and relative humidities maintained in the 2 storage rooms

Measurements	Low humidity room		High humidity room	
	Relative humidity (Per cent)	Temp., degrees F.	Relative humidity (Per cent)	Temp., degrees F.
Average of 5 weeks*	56	75	90	79
Range of weekly ave.	± 7	± 0	± 4	± 3
Range of the Absolute Max. and Min.	$\pm 11-26$	$\pm 3-2$	$+ 0-29$	$+ 10-18$

was 10.7 per cent and 10.1 per cent, respectively, for *Diplodia*; and 11.9 per cent and 15.3 per cent for *Phomopsis*. The difference in amount of *Phomopsis* present in the two rooms was nonsignificant. The amount of *Diplodia* which developed in the rooms was different to an extent which was highly significant. The conclusion is that, for 5 weeks, the high humidity in the storage room caused an increase in the amount of *Diplodia* but not *Phomopsis*.

The development of *Phomopsis* and *Diplodia* was determined in fruit that was wet after it was picked and before it was stored. Samples of 50 fruits of the four varieties were soaked in water for periods varying from 5 to 30 minutes. At the end of 3 weeks in storage there were no significant changes in the numbers of fruits rotted by the two fungi as a result of the soaking. In 15 experiments an average of 10.07 fruits were rotted by *Phomopsis* among the soaked fruits and 7.0 among the fruits not soaked, while 7.0 fruits were rotted by *Diplodia* among the soaked fruits and 5.0 among those not soaked. The least differences necessary for significance were 3.26 for *Phomopsis* and 2.43 for *Diplodia*.

Gassing with ethylene for 48 hours had no significant effect on development of *Phomopsis* in stored fruit of the four varieties, but the *Diplodia* increased by an appreciable amount. In 50 fruit samples in 25 experiments the average number of fruits rotted by *Phomopsis* was 6.9 for gassed fruits and 5.5 for checks, and the least difference necessary for significance was 1.94. The average number rotted by *Diplodia* was 13.9 for the gassed fruits and 6.0 for the checks, the least difference necessary for significance being 3.31. An F value of 22.4 was highly significant.

EFFECT OF DIPHENYL WRAPS

In an experiment arranged to test the action of diphenyl wraps in controlling stem-end rot on oranges in various combinations of manner of picking and gassing, information was collected relative to the rate of development of both *Phomopsis* and *Diplodia*. The fruit was not subjected to a packinghouse process and was stored at 75° F. at about 75 per cent relative humidity. The various treatments and results obtained by analysis of the data at the 3-week period for variances are in figure 6. The treatments were arranged in the order of their rank of importance in allowing *Phomopsis* to develop. The least difference necessary for significance at the 5 per cent level is 7.5 per cent. It is evident that the wraps were not effective in controlling *Phomopsis*. In all cases there was a significant lowering of the incidence of *Phomopsis* in pulled fruit as compared with clipped fruit. A comparison of treatments 9, 10, and 6 to determine the difference in amount of *Phomopsis* caused by various methods of removing the calyxes shows that there is no difference. In this experiment gassing had no effect on *Phomopsis*.

The development of *Diplodia* in the same lots of fruit and the results obtained in these data are shown in figure 7 where 8 per cent is the least

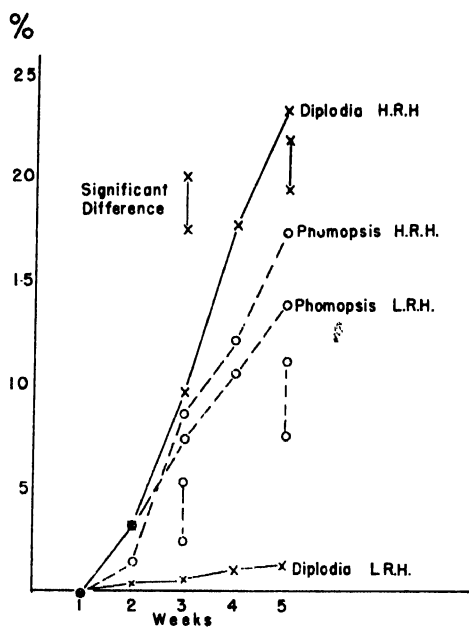


Figure 5

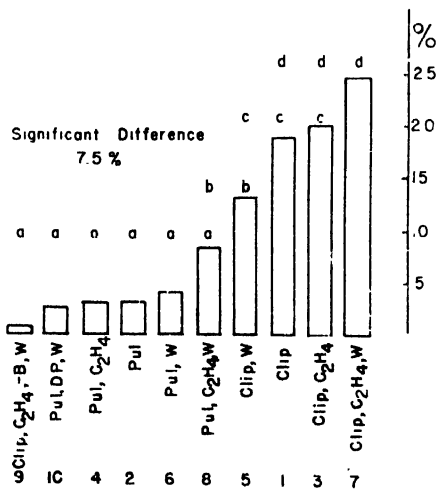


Figure 6.

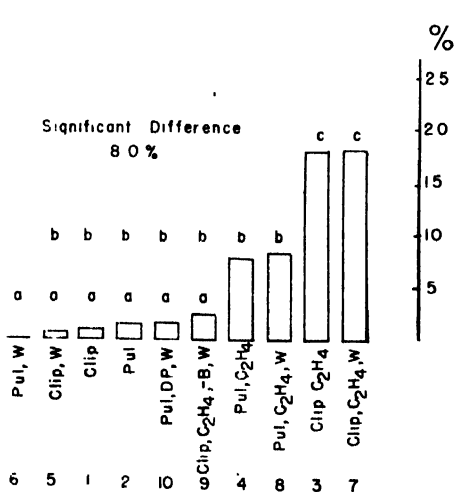


Figure 7.

FIG. 5. Rate of development of *Phomopsis* and of *Diplodia* on fruit in storage rooms having 90 per cent relative humidity (H.R.H.) or 56 per cent relative humidity (L.R.H.). Least difference necessary for significance for *Phomopsis* at 3 weeks, 11.9 per cent; at 5 weeks, 15.3 per cent; for *Diplodia* at 3 weeks, 10.7 per cent; at 5 weeks, 10.1 per cent.

FIGS. 6 AND 7. Development of *Phomopsis* (Fig. 6) and of *Diplodia* (Fig. 7) during 3 weeks in storage after various fruit treatments: Fruit picked by clipping (Clip) or by pulling (Pul); All buttons left on (+ B) or calyx removed by reamer in drill press (DP); Fruit gassed with ethylene for 48 hrs. (C₂H₄); Individual diphenyl wraps on fruit (W).

difference necessary for significance. The wraps caused no difference in the amount of *Diplodia* present during 3 weeks in storage. When the fruit was not gassed there was no difference between pulled and clipped fruit. When the fruit was gassed, buttons left on, and fruit wrapped or unwrapped, there was less *Diplodia* in the pulled than in the clipped fruit. Gassing increased the amount of *Diplodia* in all cases except where the fruit was pulled and not wrapped.

SUMMARY

From approximately 4,000 isolations made from 850 separate samples of 50 fruits each, records were kept of the incidence of *Phomopsis citri* and of *Diplodia natalensis* as they occurred with various treatments. The fruit harvest was spread over the entire picking season and included the four main varieties of oranges, Hamlin, Parson Brown, Pineapple, and Valencia.

The samples were subjected to such treatments as: debuttoning, gassing with ethylene, soaking in borax, wrapping in diphenyl-impregnated paper, decalxying by various methods, storage in high and low relative humidity, and various combinations of some of these treatments. The experiments were set up in such a manner that the data could be subjected to statistical analysis.

The 5 per cent level was chosen as the level of significance and with each experiment the least amount necessary for a significant difference between treatments was determined.

There was no correlation between the occurrence of *Phomopsis* and *Diplodia* in the same fruit.

The incidence of both *Phomopsis* and *Diplodia* was reduced by debuttoning, and by treatment with borax before, or before and after, gassing. Removal of only the calyx, not the entire button, reduced *Phomopsis* but not *Diplodia*. Gassing with ethylene did not control the fungi, and the incidence of *Diplodia* actually increased after gassing.

Borax alone reduced the incidence of *Diplodia* but not that of *Phomopsis*. Borax applied to gassed fruit, either before, or before and after gassing, also reduced the incidence of *Diplodia*.

Wetting the fruit after picking had no effect on *Phomopsis* or *Diplodia*. However, a high relative humidity during storage increased the amount of *Diplodia*.

Storing fruit in diphenyl treated wraps did not affect either of the fungi.

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BACTERIAL LEAF BLIGHT OF BIRD'S-NEST FERN

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Bird's-nest fern (*Asplenium nidus* L.) is grown on a large scale in commercial greenhouses in the San Francisco Bay region of California.

The writers' preliminary report on the bacterial disease of bird's-nest fern¹ is, apparently, the first record of a bacterial disease of ferns.

Bird's-nest ferns require two years to produce and are maintained in a constantly moist substratum in greenhouses in which high humidity and air temperature constantly prevail. There may be a rapid spread of the disease, often resulting in an epiphytotic. Losses to individual growers have in some seasons exceeded ten thousand dollars.

The disease starts as small, water-soaked, translucent spots on any part of the frond, but usually on the upper surface (Fig. 1). Infection may also occur through the water pores on the apical end of the frond. Under warm and humid conditions, the spots enlarge rapidly, soon involving the whole frond. Frequently, the bacteria invade the crown and quickly kill the plant. Necrosis of one or more fronds ruins the symmetrical appearance of the plant and thus renders it unsaleable.

The causal organism was readily isolated by macerating small bits of diseased tissue taken from the advancing margin of a lesion in sterile distilled water and plating out in potato-dextrose-peptone agar, on which the bacterium developed promptly and luxuriantly.

The pathogenicity of the isolated organism was proved in the following manner. Two isolates, one from an infection on the upper part of a leaf, and another from an infected crown, were grown on potato-dextrose-peptone agar slants at 28° C. After 24 hours, the growth was washed off with sterile distilled water (and poured) into a sterilized DeVilbiss atomizer. Small, healthy, bird's-nest ferns, grown in pots and tested for any chance infection by prolonged incubation in a moist-chamber, were inoculated. Before inoculation, the ferns were held in a large, glazed, moist-chamber with high humidity (90 per cent) for 24 hours, approximating that used by nurserymen. After spraying with the inoculum, the plants were returned to the tightly closed moist-chamber. The air temperature in the greenhouse fluctuated from 80° to 90° F. The first symptoms of the disease appeared as minute, translucent dots on the lower surface of fronds after three days. They increased rapidly, soon forming the large, translucent areas typical of the disease. When the ferns were kept in the moist-chamber 7 to 10 days fronds 3 inches in length were destroyed. When the ferns were transferred to a dry atmosphere the disease was promptly inhibited and there was little or no spread unless overhead watering was practiced. The check plants

¹ Ark, P. A., and C. M. Tompkins. Bacterial diseases of plants in California in 1945. U. S. Dept. Agr., Pl. Dis. Repr. 30: 28-29, 1946.

held under identical conditions and sprayed with sterile distilled water in a separate moist-chamber, remained healthy. Reisolations from sprayed plants yielded isolates which were identical with those used in the bacterio-

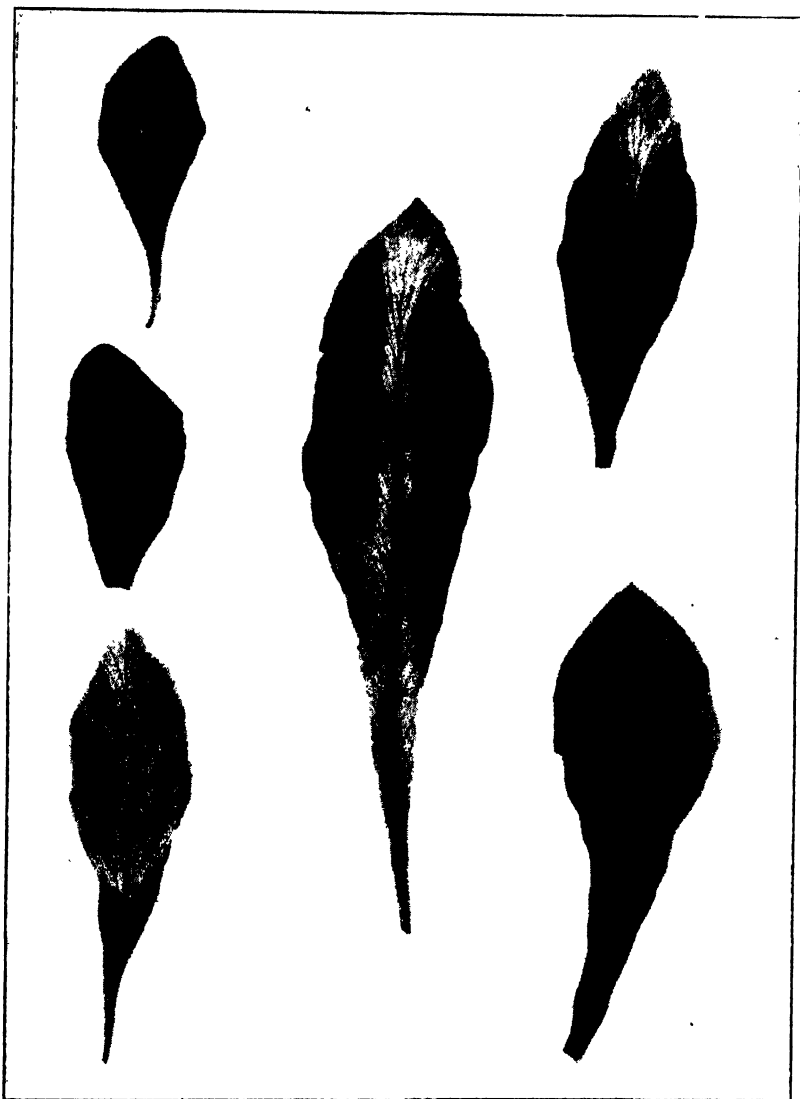


FIG. 1. Bacterial leaf blight of bird's-nest fern, *Asplenium nidus* L. Different stages in the development of the light, water-soaked lesions on the leaves. At lower right, bottom row, healthy leaf.

logical studies and produced, on inoculation, typical symptoms of the disease.

Six different isolates of proved pathogenicity and three reisolates were used in determining the bacteriological characters of the pathogen. Stand-

ard media and technique were used as described by the Society of American Bacteriologists.²

The bacterium is a short rod, $1.2-2.4\ \mu \times 0.3-0.5\ \mu$; Gram-negative; motile by means of 1 to 3 polar flagellae. On potato-dextrose-peptone agar plates, the organism grows rapidly, forming smooth, entire, flat colonies which rapidly increase in size. On beef-extract-peptone agar slants, the growth is grayish-white, with fluorescence in the medium. On potato-dextrose-peptone agar slants, growth is rapid, heavy, strongly grayish-white, butyrous; the medium darkens with time. Nutrient broth (beef-extract-peptone) is turbid within 24 hours; no pellicle³; crystals are observed with age. There is no curdling of skimmed or litmus milk. In litmus milk, a white precipitate is observed on long incubation. Indol is not formed in Dunham's solution. Ammonia is formed in peptone-glucose-dipotassium phosphate media as suggested by Hansen.³ Nitrates are not reduced to nitrites. H_2S is not formed in Bacto lead acetate agar. In tubes of Fermi's solution, previously sterilized by autoclaving for 15 minutes at 15 pounds pressure, to which the following sugars, previously sterilized by filtering through UF sintered filters, and inverted Dunham fermentation tubes were added, acid but no gas is formed from the following carbohydrates: dextrose, sucrose, maltose, arabinose, xylose, glycerin, galactose, and fructose. Very slight acidity develops in lactose broth on long incubation. Very scanty growth occurs in raffinose broth, and no acid. Good growth occurs in the following synthetic media: Soc. Amer. Bact., Fermi's, Cohn, and Uschinsky. Starch is not hydrolyzed. Gelatin is liquefied. The organism is a facultative anaerobe. Thermal death point, 50°C . The organism develops at temperatures from 1° to 34°C ., with optimum growth at $22^\circ-30^\circ\text{C}$.

The name *Phytomonas asplenii* n. sp. is proposed.

Control of this disease is entirely dependent upon sanitation and good cultural conditions. The pathogen can be introduced into the brick-dust medium used for propagation purposes on fern spores taken from old fronds of infected plants. Therefore, in propagating, spores should be taken only from healthy plants. Where the disease is present it is important to destroy all plants with symptoms and to grow the healthy plants under drier conditions. The brick-dust and ratsnest (a natural forest compost), flats, and pots should be steam-sterilized.

When the prothallia and young plants are being transplanted, they should be handled with forceps which are frequently flamed in alcohol.

Tests in several commercial greenhouses in San Francisco over a period of two years proved conclusively that sanitation and improved cultural conditions will yield healthy bird's-nest ferns.

² Society of American Bacteriologists. Manual of methods for pure culture study of bacteria. Leaflet II. Preparation of media. 9th ed. 1944.

³ Hansen, P. A. The detection of ammonia production by bacteria in agar slants. Jour. Bact. 19: 223-229. 1930.

SUMMARY

1. A bacterial disease of bird's-nest fern causes serious losses in greenhouses in central California.
2. A bacterial organism isolated from the advancing margins of the lesions proved pathogenic upon inoculation and was readily reisolated.
3. The causal organism is described and the name *Phytomonas asplenii* n. sp. is proposed. The colonies are grayish-white. The organism is motile by flagellae attached to one pole.
4. Control measures consist of strict sanitation involving steam-sterilization of the brick-dust and ratsnest media, flats, and pots, the use of sterile forceps in transplanting, and avoidance of excessive watering and too high humidity in the greenhouses.

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WITCHES' BROOM OF ALFALFA IN NORTH AMERICA¹

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(Accepted for publication June 10, 1946)

INTRODUCTION

The witches' broom disease of alfalfa (*Medicago sativa* L.) has been recognized in the United States since 1925. A disease of the same name and symptomatology also occurs in Australia where it has been studied in detail by Edwards (5, 6). The disease in America has received very little attention because of its sporadic occurrence and minor importance. Recently, however, it has become a serious problem in localized areas of Washington (15) and of British Columbia (2) in Canada. Edwards showed that the Australian witches' broom is a virus disease, transmissible by grafting but not by mechanical means. The present studies were undertaken to clarify the relationship between the American and Australian alfalfa witches' brooms and to further investigate methods of transmission.

HISTORY AND DISTRIBUTION

Most of the previous references to alfalfa witches' broom in America are records of occurrence only, with occasional descriptions of symptoms. Although most reporters considered the symptoms to be indicative of a virus disease, it was not until Heald and Wellman (10) reported graft transmission in 1939 that this belief received experimental support.

The first North American record of this disease appears to have been a report by Haskell (8) of a witches' broom on alfalfa in Idaho in 1925. According to Richards (16) the disease appeared in Utah about the same time. In 1932, in a brief report, Foster (7) mentioned the occurrence of witches' broom in two widely separated areas in British Columbia. Other reports show that the disease was first found in Washington in 1934 (9) and in Alberta in 1940 (4). It has been found occasionally in the Umatilla Valley of Oregon but the history of witches' broom in that State is not known. Recently this disease has been reported from Arizona by Hoyman (11) who, in 1943, found less than 1 per cent in one of the older alfalfa plots located at the University of Arizona Farm on the Yuma Mesa. Dr. Hoyman states, in correspondence, that witches' broom was not observed in younger stands on the Mesa or elsewhere in the State.

These reports limit the known distribution of witches' broom to the area between the Cascade and Rocky Mountains except for an eastern extension

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of the disease into Alberta. A single specimen of a witches' broom on alfalfa has been reported from Vancouver Island (7). Within this general area witches' broom has some very interesting features of distribution and occurrence. Although the disease is widely scattered in the Northwest, it has reached serious proportions in only 3 or 4 relatively isolated areas. The development of the disease in Utah, Idaho, Washington, and British Columbia has a rather consistent pattern.

The Utah outbreak was the first to occur. According to Richards (16) the disease was first seen in Salt Lake County in 1925. By 1928 it was considered an important factor in reduction of stands. Dr. Richards reports, in recent correspondence, that although the disease at one time could be found in older fields to the extent of 60 to 65 per cent of the plants, it has now almost entirely disappeared, being found only along ditch banks and fences. This reduction is correlated with short rotations adopted for bacterial wilt control but it seems unlikely that so striking a control of witches' broom could be obtained in this manner.

Dr. E. C. Blodgett has recently furnished the author with a summary of the witches' broom situation in Idaho. Following the original record of the disease in Elmore County in 1925 no further observation of witches' broom was made until Blodgett noted it along the Snake River in the same county during a plant disease survey in 1944. Although the survey included most alfalfa areas of Idaho, the disease was not found elsewhere in the State. Blodgett found many fields in the Elmore County area wherein infections ranged from a trace to 50 per cent. The disease is important in the area because of the resulting reduction of stands.

In Washington, scattered infections have been noted in Walla Walla, Benton, Franklin, Yakima, Grant, and Okanogan counties, but except for moderate severity in small plots at Prosser (Benton County) the disease is important only in the Methow Valley area of Okanogan County, where it was first reported in 1934 (9). In 1939 Heald and Wellman (10) reported infections in this area ranging from 25 to 60 per cent. Later surveys by the author show that the disease continued to increase during the next 3 years. Infections during those years reached as high as 80 per cent with accompanying stand reductions. Some growers reported having to plow and reseed fields in as short a time as 3 years because of witches' broom infection.

The Methow Valley is narrow and isolated from the more open alfalfa areas in central Washington. The original outbreak of witches' broom was at the upper end of the valley near Mazama. Since then it has spread to involve almost all the alfalfa areas of the valley to a point about 30 miles below Mazama. Since 1942 there is evidence that new plantings in the center of the original diseased area are being less severely attacked, although infections in more recently invaded areas are still at a high level. Young stands in the Mazama area now have only scattered infections whereas in previous years growers despaired of maintaining stands in these fields be-

cause of the severity of the disease. The topography of the area is very irregular and it is, therefore, difficult to decide whether or not there is a "crest" of infection moving outward from the original point of occurrence. The rate of spread down the valley during the last 3 years has been slow, and has now reached a natural barrier of rough, uncultivated territory which may effectively isolate the disease from the other alfalfa areas of the Columbia Valley.

The information available on the distribution of witches' broom in British Columbia (2) shows that infections have built up in the Nicola Valley comparable to the Methow Valley area. Here, again, severe disease development has been strikingly localized.

Witches' broom, therefore, has a 20-year history of persistent localization of severe outbreaks. The natural recession of the disease in Utah and the indications of the same phenomenon in Washington are hopeful signs that the same may occur in other areas. The factors responsible for witches'-broom outbreaks must be common to these areas but not general within the known distribution range. These factors are not known, but it seems most likely that they are concerned with the population trends of insect vectors.

A witches' broom on sweet clover has been reported in Alberta (3) but has not been found in Washington even in areas where alfalfa is seriously affected. Very similar symptoms, however, occur on red clover and White Dutch clover in Washington and have been seen occasionally also on black medic (*Medicago lupulina* L.). No hosts other than alfalfa have been reported for the disease in other states or in Australia.

SYMPTOMS AND EFFECTS

Witches' broom of alfalfa is a disease which slowly modifies the appearance of affected plants during the course of one to several seasons. Consequently a wide range of symptoms is encountered in the field. In the typical or advanced stage of infection the plants are severely dwarfed and bunchy (Fig. 1, B). This is due to the excessive development of short spindly shoots from all parts of the crown and from axillary buds along the stems. The plants have a yellowish cast as a result of marginal chlorosis of the leaves and a general pale-green color of the tiny succulent stems. Normal alfalfa leaflets may be described as oblong or elliptic-oblongeolate with spinose denticulations toward the apex. Leaflets on affected plants are definitely smaller, obovate to cuneate, usually lack apical denticulations, and are frequently wrinkled or puckered as shown in figure 2, A. Usually, witches'-broom-infected plants are erect and compact but may develop a prostrate habit of growth as the tops approach maturity. Diseased plants are easily recognized in the field, even from a considerable distance, especially if the stand is thin or newly cut.

The first symptoms of infection usually are seen after new growth commences following cutting. Plants producing symptoms for the first time

invariably have the same degree of infection over the entire plant. There may be little or no dwarfing but the plants can be recognized immediately by

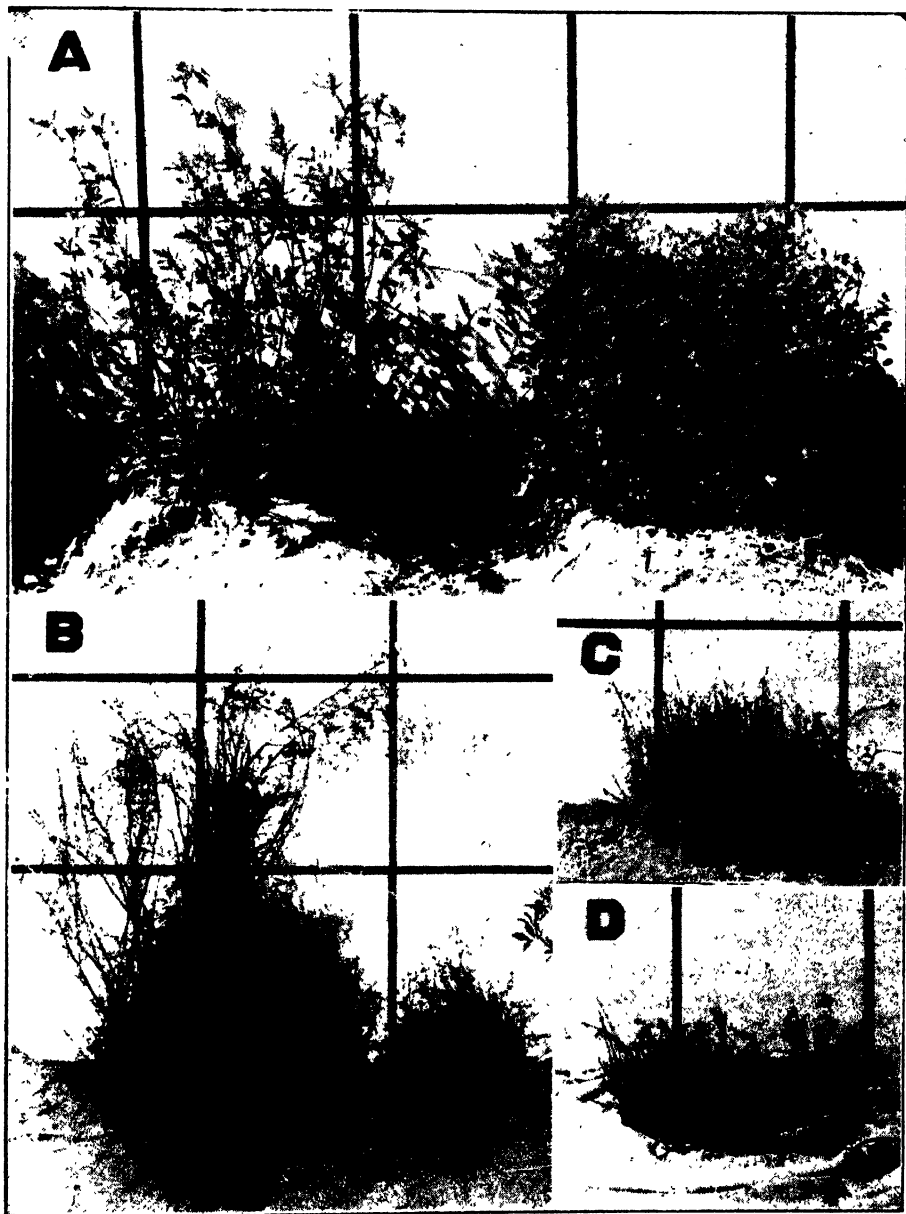


FIG. 1. Alfalfa witches' broom. A. Early stage of infection (right) contrasted to healthy plant (left). B. Two severely diseased plants, the larger one with tufts of witches' brooms on previously healthy stems. C. Advanced stage of witches' broom. D. Severe symptoms of bacterial wilt for comparison. All photographs by H. P. Singleton.

the greatly increased number of stems and erect habit as well as by a slight marginal chlorosis of the younger leaves. After the next cutting the new

growth will usually be decidedly dwarfed and the leaflets much reduced in size. Diseased plants become progressively stunted after each cutting and the number of stems is increased. Several hundred spindly stems are common on infected plants while severely infected crowns have been found with as many as 3,000 very fine, densely matted stems. Such plants can scarcely be recognized as alfalfa because of severe dwarfing and an accompanying reduction of leaflets to tiny, bract-like structures on the ends of the petioles.

While affected shoots usually originate from the crown they may also

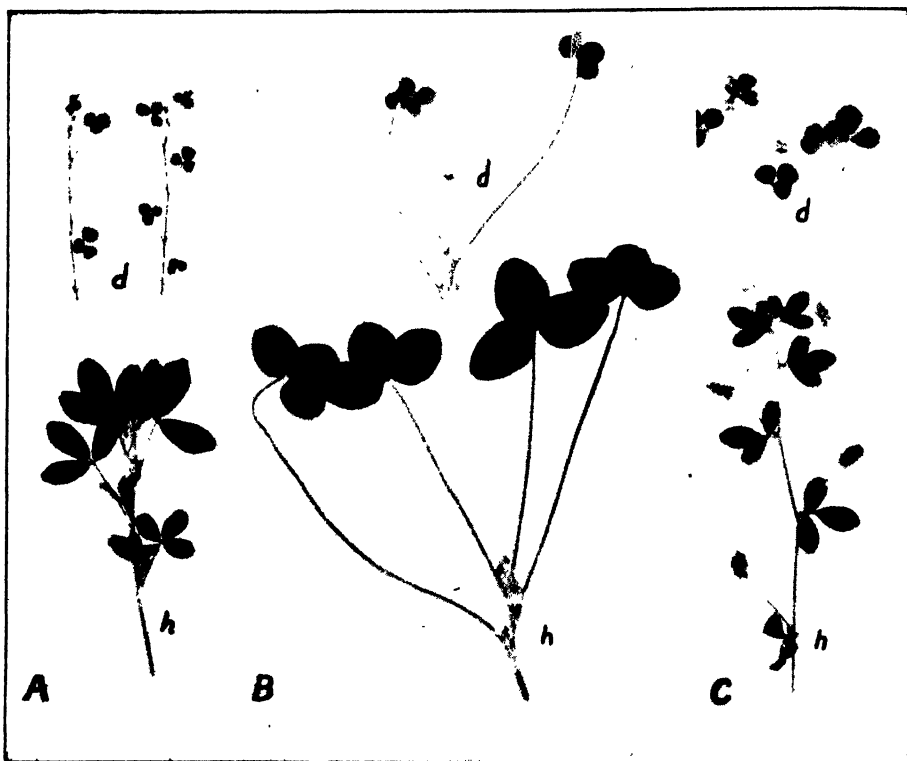


FIG. 2. Symptoms of alfalfa witches' broom obtained by graft transmission. A, on alfalfa; B, on red clover; C, on black medic (*M. lupulina*). Healthy shoot (h), diseased shoot (d).

appear in tufts at the nodes on previously healthy stems. This condition occurs on newly infected plants when the old, symptomless growth is not removed by harvesting. A general view of such a plant is shown in figure 1, B.

Witches' broom causes a definite decrease in yield because of the dwarfing but the chief source of loss is the reduction of stands by the early death of diseased plants. Plants die during the winter, which suggests that the infected plants are so weakened by the constant proliferation of new shoots that they readily succumb to low temperatures. Data were obtained on death rate of diseased plants in a row-planted nursery at the Irrigation

Branch Experiment Station, Prosser, where it was possible to follow the course of the disease in individual plants.

During a 3-year period 131 newly infected plants were observed during one winter, 54 were observed through 2, and 17 through 3. In the first group 50 per cent of the plants died during the first winter. In the second group 39 per cent died the first winter and 81 per cent were dead after the second. In the third group the rate of death was 35, 59, and 90 per cent at the end of 1, 2, and 3 winters, respectively. Thus, in these cases, almost half the infected plants died the first winter and only 10 per cent survived three winters. The decrease in number of healthy plants in the same nursery during the same 3-year period was from 2,507 to 2,078, an average annual death rate of approximately 6 per cent.

Seed production is almost entirely inhibited in diseased plants. The few flowers which are produced are usually in groups of two or three instead of in many-flowered racemes typical of normal plants. The blossoms are smaller and paler than the normal and rarely produce seed. The small quantity of seed collected from infected plants has been poor in appearance but has germinated well.

TRANSMISSION EXPERIMENTS

Mechanical Transmission Tests

Neither Edwards (6) nor Heald and Wellman (10) were able to transmit witches' broom by mechanical inoculation methods. Edwards used various techniques involving the use of cotton swabs, pin pricks, and hypodermic needle injections into stems and roots. The method used by Wellman was probably the one recommended by Jones (13) as being sometimes effective when other methods fail. This consists of using macerated diseased tissue on a swab as inoculum and dusting carborundum powder on the leaves to be inoculated.

This method was used by the author to inoculate 250 plants but no infection resulted. During the five years of this study many healthy and diseased plants have been handled in the greenhouse without regard to the possibility of accidental mechanical transmission, yet none is known to have occurred. In unsuccessful grafting attempts where diseased scions have remained alive for as long as 10 days before withering, there has been no transmission of the disease. It therefore seems unlikely that mechanical transmission can occur and certainly there is little possibility of the disease being spread in the field by the mower or other mechanical means.

Graft Transmission Tests

Both Edwards and Heald and Wellman were successful in transmitting witches' broom by grafting. However, since it could not be assumed that the Australian disease was the same as the one in the United States, and since the work of Heald and Wellman was incomplete, grafting tests were repeated on a more extensive scale. Edwards used a wedge- or veneer- root-

grafting technique yielding about 12 per cent successful grafts. Heald and Wellman also used various types of root grafting with methods found to be satisfactory by Weimer (17) in grafting studies on alfalfa dwarf.

Preliminary root-grafting tests were tried but because of a low percentage of successful grafts, the method was abandoned in favor of a modification of the shoot-grafting technique. Because of the slenderness and succulence of witches'-broom-infected shoots the usual cleft-graft method as used on more robust herbaceous plants was not satisfactory. The chief change necessary in using the method was to keep the delicate grafts in a humid atmosphere in a glassed humidity chamber for at least 10 days after grafting. The unions were carefully but firmly bound with fine thread which could later be removed. After considerable practice it was possible to obtain as high as 75 per cent successful grafts by this method.

TABLE 1.—*Time required for sufficient tissue union in alfalfa grafts to allow transfer of the witches'-broom virus*

Duration of graft	Transmission results	
	Total plants	Plants infected
<i>days</i>	<i>number</i>	<i>number</i>
5	5	0
10	5	0
12	5	2
16	5	3
20	5	4
25	4	4

In all cases where diseased scions became established on healthy alfalfa plants, transmission of witches' broom was obtained. In one typical experiment with 5 root-approach grafts the disease was transmitted in 118 days while with 23 shoot grafts it was transmitted in an average of 61 days. The results with the root grafts are in accord with those of Edwards who reported a latent period of the virus in the plant of from 4 to 5 months. The delayed transmission with the root grafts may be due in part to a greater length of time required for adequate tissue union.

The actual latent period between inoculation and symptom expression is difficult to determine in the case of alfalfa witches' broom because of indefinite early symptoms. Thus, the latent period ranged from 32 days to 137 days in the case of shoot grafts, a variation which appeared to be related to growth rate of the plants, faster growing plants producing the earliest symptoms. Since these figures also include the time necessary for sufficient tissue union to allow virus transfer, a scion-removal experiment was performed to determine this interval. Thirty young alfalfa plants were grafted by the shoot-graft method with scions affected with the disease. Five days after grafting the scions were removed from 5 of the plants and from groups of 5 others at intervals of 10, 12, 16, 20, and 25 days. All plants were kept in the greenhouse for 4 months before final records were taken.

Table 1 shows that virus transfer commenced between the 10th and 12th day and had taken place in all plants in from 20 to 25 days. Thus, under the conditions of these tests the actual latent period of the virus in the plants averaged approximately 40 to 50 days.

Studies of Host Range by Means of Grafting

The report of witches'-broom symptoms on sweet clover in Alberta and the occurrence of similar symptoms on red clover, White Dutch clover, and black medic in Washington led to attempts to determine the leguminous host range of the virus by means of cross-grafting. This method has been used successfully with solanaceous plants but has not been very successful in other families beyond generic limits.

In the fall of 1941, some preliminary grafts were made on 2 species of sweet clover (*Melilotus alba* and *M. officinalis*) using witches'-broom-infected alfalfa scions. Eventually 12 scions were established on plants of *M. alba* and 7 on *M. officinalis*. These plants were observed in the greenhouse for one year. During this time the growth of scions on *M. alba* was stunted, but the unions appeared to be sound. No symptoms of witches' broom appeared on the sweet clover and back grafts to alfalfa indicated the absence of virus. The stocks of *M. officinalis* died during the year without any evidence of virus transmission.

During the winter of 1942-43 numerous cross grafts from diseased alfalfa to various species of legumes were attempted. Although many of these grafts failed to survive longer than the initial humidity treatment, eventually 87 apparently successful grafts were obtained on 12 different species of legumes. At the same time a few additional grafts were made on alfalfa as a check on the technique.

Although the numerous alfalfa scions on different legume species seemed to be well established, it eventually became evident that all alfalfa scions on genera other than *Medicago* had failed to make union but were being maintained on their own adventitious roots which had become anchored in the pith tissue of the stock. The final results both in scion rooting and transmission are summarized in table 2. The fact that the scions were producing roots rather than uniting with the stock became evident when living alfalfa scions were noted on red clover stems that had died. On red clover the scion roots were very fine and could be mistaken for vascular strands of the clover, in other cases the scions produced such vigorous roots that the stock stem was eventually split and shredded and the roots became anchored in the soil. In the case of the one surviving scion grafted on bean there was no external evidence of rooting even 3 months after grafting and although the scion was not thrifty it was still living. At the end of this period the bean stem was cut open revealing a single, stout alfalfa root about three inches long extending through the pith tissue of the stem but not reaching the soil. Although rooting of scions was not common on *Medicago* species in this test, later grafts with alfalfa resulted in several

cases. Microscopic examination of many grafts of diseased scions on healthy alfalfa revealed a frequent tendency for root primordia to form, but root development appeared to be checked by satisfactory tissue union. In one case scion rooting was accompanied by sufficient tissue union for virus transmission. Since healthy alfalfa scions were not used in any of these grafts it is not known what effect the virus may have in stimulating the rooting tendency. However, back grafts with apparently healthy scions of red clover and white sweet clover onto healthy alfalfa also gave the rooting response without tissue union.

These results show that scion survival in cross grafts with the legumes used does not constitute sufficient evidence for tissue union. Consequently use of the method for studying virus host range in this family does not appear promising except within genera.

TABLE 2.—Results of cross-grafting experiments with witches' broom infected alfalfa scions on various species of legumes

Stock species	Plants with living scions			Good union	Scions rooted	Virus transmitted
	After 12 days	After 30 days	After 90 days			
	no.	no.	no.	no.	no.	no.
<i>Medicago sativa</i> L.	13	10	8	6	2	8
<i>M. lupulina</i> L.	10	5	5	5	0	5
<i>M. hispida</i> Gaert.	4	4	3	3	0	3
<i>M. arabica</i> Huds.	8	4	3	3	0	2 ^a
<i>Trifolium pratense</i> L.	12	12	8	0	8	0
<i>T. fragiferum</i> L.	4	2	0			0
<i>T. hybridum</i> L.	10	8	3	0	3	0
<i>T. repens</i> L.	7	5	0			0
<i>T. incarnatum</i> L.	10	10	5	0	5	0
<i>Lotus corniculatus</i> L.	6	0	0			0
<i>Onobrychis</i> sp.	5	4	1	0	1	0
<i>Phaseolus vulgaris</i> L.	4	2	1	0	1	0
<i>Robinia Pseudo-Acacia</i> L.	7	1	0			0

^a Plants matured and died without showing disease symptoms.

The cross grafts to other species of *Medicago* were successful as evidenced by good scion growth and absence of rooting. Five grafts out of 10 attempts were obtained on *M. lupulina* with transmission of the virus in each case.

The symptoms, as shown in figure 2, C, are similar to those produced on alfalfa. Back grafts from these plants to healthy alfalfa produced the typical symptoms of the disease in the latter. With *Medicago hispida* 3 successful grafts were obtained followed by the appearance of witches'-broom symptoms in the stock after an interval of approximately 3 months. Although 3 successful grafts were obtained on *M. arabica* no appearance of symptoms was recorded prior to the death of the plants. Since this species is an annual it is possible that the grafting was done too late for symptoms to develop before the plants matured. Previous mention has been made of the natural occurrence of witches' broom on red clover and White Dutch clover.

The inability to obtain grafts between diseased alfalfa and either red clover or White Dutch clover prevented experimental proof of the relationship between the naturally occurring witches' brooms on these 3 hosts. Infected plants of these 2 clovers were used in the greenhouse for various grafting tests. No permanent grafts with witches'-broom scions of white clover were obtained on any species. Diseased red clover scions, however, were grafted to healthy red clover with transmission of the typical witches'-broom symptoms as shown in figure 2, B. Since the clover witches' brooms are found only in association with alfalfa witches' broom and since the red clover form is also graft-transmissible, it is very likely that they are all caused by the same virus.

The host range of alfalfa witches' broom, therefore, definitely includes *Medicago lupulina* and *M. hispida*, probably includes *Trifolium repens*, *T. pratense*, and according to Cormack (4), also includes *Melilotus* sp.

Insect Transmission Tests

The only previous attempt to find an insect vector for alfalfa witches' broom was by Edwards (6). In most of his experiments mass collections of insects from alfalfa fields were allowed to feed at will on both healthy and diseased plants under large cages. No transmission was obtained with any of the insects used. Since field observations suggested the presence of an active insect vector, tests were begun in Washington in 1941 with sucking insects common in alfalfa fields. A report has already been made on preliminary evidence that the leafhopper, *Platymoides acutus* Say, can transmit this disease (14). Since the details of the tests were not published, they are reported here.

In the exploratory vector tests, mass collections of suspected species were caged on infected alfalfa plants for a short feeding period after which they were segregated into species groups and transferred to healthy plants. No attempt was made to obtain identification of insects giving negative results since such tests were not considered conclusive.

In the course of several hundred tests with sucking insects one case of transmission occurred where *Platymoides acutus*³ was used. In this case a large, but undetermined, number of leafhoppers of this species was caged on an infected plant from May 26 to June 2, 1943, after which they were transferred to a healthy plant for 12 days. At the end of this time 7 insects were still living. Witches'-broom symptoms were first considered definite on August 14, approximately 2 months after inoculation.

A second experiment was then begun using larger cages, in which one infected plant and 4 to 6 healthy plants were placed. Field collections of the suspected species were added to some of the test cages from time to time during August and early September, while other cages were held as checks. By the end of October all the insects had died and the plants were removed to the greenhouse bench for observation.

³ Identification of this insect was kindly made by Dr. R. H. Beamer, University of Kansas.

No symptoms appeared on these plants until early in February, 1944, when 2 plants were recorded as suspicious. Symptoms continued to develop on these and other plants until finally, by May 15, eighteen of the 29 surviving test plants were definitely affected with witches' broom. None of the 6 check plants became diseased and no infections appeared on uncaged healthy alfalfa that had been held in the same greenhouse during the entire period of the test.

A third series of tests with the same leafhopper was carried out during the summer of 1944. Small cages to fit 6-inch pots were used. Each pot contained from 5 to 10 alfalfa seedlings grown from seed under the cages. Details of each test and the transmission results are shown in table 3.

TABLE 3.—Results of witches'-broom transmission tests on alfalfa seedlings with the leafhopper *Platymoides acutus* Say, during the summer of 1944

Pot No.	No. of alfalfa seedlings	No. of insects		Source of insects	Feeding period	No. of plants infected
		Adults	Nymphs			
1	5	10	1	Diseased plt. ^a	21 days	4
2	9	4	1	do	indef.	4
3	5	0	3	do	21 days	3
4	6	1	20	Field coll. ^b	indef.	3
5	6	5	25	do	indef.	0
6	6	1	15	do	17 days	4
7	5	0	3	do	17 days	0
8	6	1	2	do	18 days	0
9	4	0	3	do	18 days	0
10	5	Check—no insects				0
11	4	do				0
12	5	do				0
13	5	do				0
14	6	do				0
15	8	do				0

^a Insects caged on an infected plant for at least 10 days.

^b Insects taken from an alfalfa nursery with approximately 50 per cent witches' broom.

The percentage of transmission obtained in the various cages of the third test appears to bear some relation to the number of insects. The mortality rate was high in all cages and may account for the poor transmission recorded in cages where only a few insects were used.

The consistent transmission of witches' broom in all tests with *Platymoides acutus* and the absence of transmission in the check cages establishes this insect as a vector of the virus. Certain field observations, however, suggest that it may not be the most important natural vector. The insect could not be found in the Methow Valley during 2 visits there in the summer of 1944. These searches were made in June and August, when the insect could be found in the Yakima Valley, and included sweepings in fields very severely infected with witches' broom. Furthermore, field records at Prosser indicate a peak of new infections early in the season whereas the peak of population of *P. acutus* occurred in late August. Even though this species may not be the chief vector its widespread occurrence provides a

possible means by which witches' broom could become established in new areas.

Dodder Transmission Tests

The ability of various species of dodder (*Cuscuta spp.*) to act as transmission bridges for certain viruses has been demonstrated by Bennett (1), Johnson (12), and others. Since dodder was found generally distributed in alfalfa fields in the Methow Valley, the possibility that dodder might transmit the witches'-broom virus was investigated.

Field-grown *Cuscuta campestris* Yunk. was established in the greenhouse on 9 rooted cuttings of witches'-broom-infected alfalfa in small pots. After the parasite had made sufficient growth the dodder strands from each plant were trained across to a healthy alfalfa plant in an adjacent pot. Nine such pairs were established each having several well connected dodder bridges. The period of union varied from 17 to 62 days after which the dodder was removed and the healthy plants kept for observation. None of these plants subsequently developed symptoms of witches' broom, which indicates that this species of dodder is not a factor in transmission of the virus.

Seed Transmission Tests

To test the possibility of seed transmission of the witches'-broom virus, collections of seed were made from infected plants. These plants rarely set seed and it was possible to collect only a very small amount for this purpose. In 2 different tests, 488 plants were grown from this seed and kept for at least one year in a nursery. None of the plants had any evidence of witches'-broom infection.

COMPARISON OF AMERICAN AND AUSTRALIAN WITCHES' BROOM

No positive evidence has been obtained to show that the witches' broom described by Edwards in Australia differs from the one in the United States. The symptoms appear to be identical and the transmission experiments show similar agreement where positive data are available for both diseases. Edwards reported a phylloid condition of blossoms on infected plants which he considered to be part of the symptom picture. A similar phyllody has been observed in Washington on both witches' broom and healthy plants and is therefore believed to be unrelated. Edwards also reports occasional recovery of diseased plants when transplanted to the greenhouse, a phenomenon that has not been observed here. Those possible differences require further study before they can be evaluated. In the meantime it is justifiable to assume that the 2 diseases are the same.

SUMMARY

Alfalfa witches' broom is a virus disease occurring in Western United States and adjacent Canada. It appears to be the same as the alfalfa witches' broom prevalent in southern Australia. Comparison of known features of the two forms of disease shows no positive differences.

This disease has become serious in isolated areas of the Pacific Northwest but has not spread widely within this area since its first appearance 20 years ago. The history of the disease indicates sporadic outbreaks followed by natural recession.

Affected plants are decidedly dwarfed and have a dense proliferation of shoots from the crown. Death follows in from one to three years. With heavy infection, stands are rapidly reduced.

Witches' broom occurs naturally on alfalfa, red clover, and White Dutch clover. It has also been reported on sweet clover in Alberta. Grafting tests have resulted in artificial infection of *Medicago lupulina* and *M. hispida*.

The virus has been transmitted by both root and shoot grafting and by means of the leafhopper *Platymoides acutus* Say. Negative results were obtained in efforts to transmit the witches'-broom virus by mechanical means, by dodder, or through the seed.

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PHYTOPATHOLOGICAL NOTES

An Undescribed Coryneum on Diseased Italian Cypress.—An undescribed species of *Coryneum* was found on an Italian cypress (*Cupressus sempervirens* L.) from the vicinity of Montgomery, Alabama. Although dead terminal buds, leaves, and branchlets of the cypress gave evidence of a diseased condition, the pathogenicity of the fungus has not been investigated. The fruiting bodies of the fungus appear as small black pustules scattered sparingly on the leaf surfaces of the host or occasionally coalescing on the leaf margins. No perfect stage was observed.

Coryneum asperulum sp. nov.¹

Acervuli conspersi, atri, erumpentes, pulvinati, plerumque circulares, 50–200 μ in diam.; conidia oblongo-fusoida, asperula, typice 3-septata, olivaceo-grisea, 27–32 $\mu \times$ 5–6 μ (19.0–40.6 $\mu \times$ 3.6–6 μ), ad septa non- vel subconstricta, cellula terminali ad apicem muticum leniter attenuata, cellula basali truncata; conidiophora simplicia, brevia; pseudoparaphyses nulli.

In foliis morboris Cupressi sempervirentis, Alabama.

Acervuli scattered, black, erumpent, pulvinate, usually circular, 50–200 μ diameter; conidia oblong-fusoid, slightly roughened, typically 3-septate, deep olive-gray to dark olive-gray, 27–32 $\mu \times$ 5–6 μ (19.0–40.6 $\mu \times$ 3.6–6 μ), not or only slightly constricted at the septa, terminal cell slightly tapering to a muticate apex, basal cell truncate; conidiophores simple, short; pseudoparaphyses absent.

On diseased foliage of *Cupressus sempervirens* L.: Alabama. Type (F. P. No. 48276) in the Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, the New York Botanical Garden, and the Farlow Herbarium of Harvard University. The general structure of the fruiting body is shown in figure 1, A and B, with spores shown in C.

Twelve species of *Coryneum* have been described on conifers; of these, two occur on *Cupressus*. Wagener² reported *Coryneum cardinale* Wagener as the cause of serious stem and branch cankers on the Monterey cypress (*Cupressus macrocarpa* Hartw.) and, occasionally, on the columnar Italian cypress (*C. sempervirens* L. var. *stricta* Ait.) in California. Natural infections were observed on *C. pygmaea* (Lemmon) Sarg., *C. forbesii* Jeps., and *C. lusitanica* Mill.; and artificial inoculations extended the list of susceptible species and varieties of *Cupressus*. *Coryneum berckmanii* Milbrath, which causes a blight on the foliage and young stems of ornamental varieties of *Thuja orientalis* L., occasionally affects the columnar Italian cypress in Oregon.³ Both *C. cardinale* and *C. berckmanii* differ from *C. asperulum*

¹ The Latin description was prepared by Edith K. Cash, Associate Mycologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

² Wagener, Willis W. The canker of *Cupressus* induced by *Coryneum cardinale* n. sp. Jour. Agr. Res. 58: 1–46. 1939.

³ Milbrath, J. A. Coryneum blight of Oriental Arborvitae caused by *Coryneum berckmanii*, n. sp. Phytopath. 30: 592–602. 1940.

by having 5-septate spores. Only three of the twelve species reported on conifers belong to the 3-septate group. These are *C. bicornis* Rostr. reported

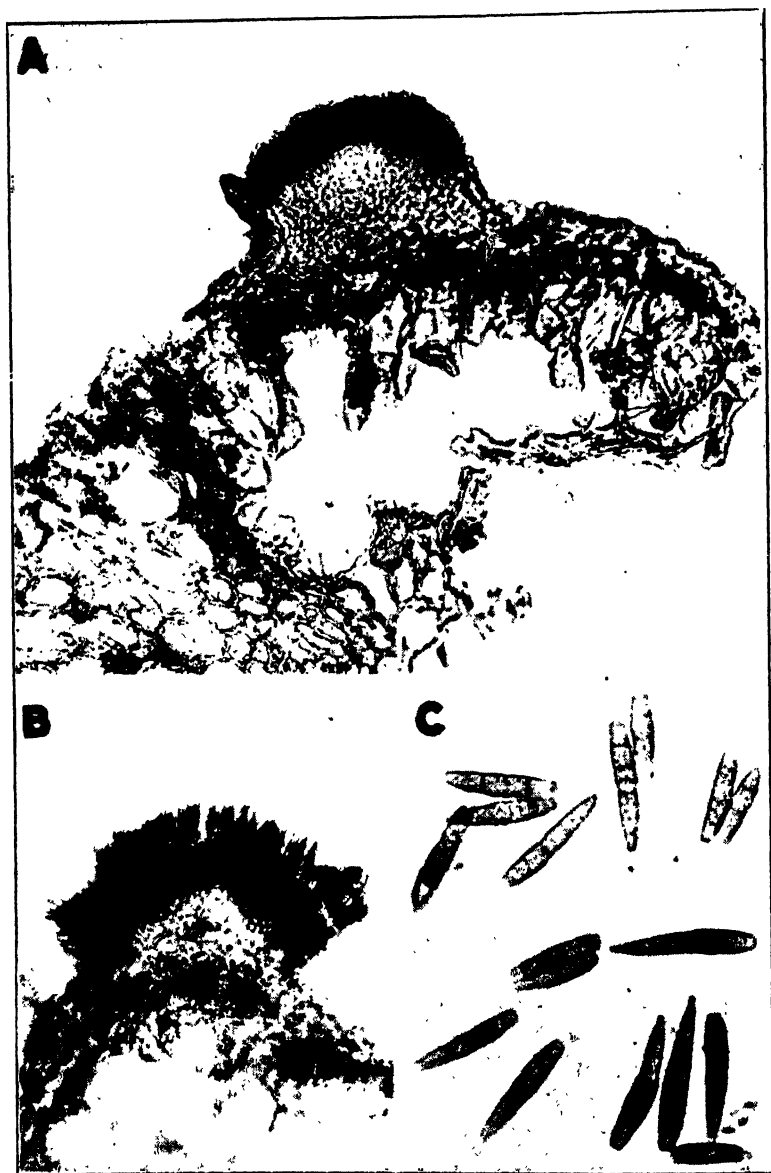


FIG. 1. *Coryneum asperulum*. A, Cross section of acervulus with spores dislodged. (Note host epidermal tissue.) $\times 225$. B, Cross section of mature acervulus with spores attached. $\times 225$. C, Mature spores. $\times 600$. Photomicrographs by M. L. F. Foubert.

on leaves of *Abies pectinata* DC. in Denmark,⁴ *C. thycolum* Vest. on leaves

⁴ Rostrup, E. Mykologiske meddelelser (VIII). Botanisk Tidsskrift 22: 254-276. 1898-99.

of *Thuja occidentalis* L. in Sweden,⁵ and *C. cinereum* Dearu. on older needles of *Pinus contorta* Dougl. and *P. murrayana* Grev. and Balf. in Oregon and Colorado.⁶ *Coryneum asperulum* differs from *C. bicornis* by having longer and more narrow conidia. Furthermore, the conidia of *C. bicornis* are curved and bifurcate. The conidia of *C. thycolum* are characterized by conic papillae at each end and are much broader than those of *C. asperulum*. The long conidiophores and shorter and broader conidia of *C. cinereum* differentiate it from *C. asperulum*. Karsten⁷ described a fungus from the needles of *Juniperus communis* L., which he placed in the closely related genus, *Erosporium*, and stated that his species, *Erosporium deflectens* Karsten, resembled *Coryneum*. Although *E. deflectens* has 3-septate conidia, the spores are much shorter than those of *C. asperulum*.—FRANCES F. LOMBARD and ROSS W. DAVIDSON, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland.

*Reduction in Yield of the Anjou Pear Caused by Wettable-sulphur Spray.*¹—Wettable-sulphur fungicides used for control of the scab fungus (*Venturia pyrina* Aderh.) at Hood River, Oregon, were reported recently² to cause russetting of the fruit and lighter green color of the foliage of the Anjou pear variety. Although early observations indicated that sulphur sprays might influence the yield of fruit, completely satisfactory experimental evidence was not obtained until 1945. A summary of the effect of fungicides upon the fruit set and fruit yield of Anjou pears for 1942 through 1945 is presented in this paper.

Plots and spray program. The plots, selected primarily for pear scab control experiments, were located in 2 different orchards. In both orchards the Anjou trees were relatively vigorous and at the peak of their productivity. The trees had been pruned to prevent interlocking of limbs and excessive shading of the lower branches. Fertilization, cultural practices, and the opportunity for pollination by other varieties were considered satisfactory for commercial crop production.

All the experimental trees received a delayed dormant application of either lime-sulphur solution or lime-sulphur plus oil emulsion to control insect pests and the overwintering scab fungus in lesions on twigs. Previous evidence had indicated that the delayed dormant sprays had no adverse effect upon fruit set.

⁵ Vestergrén, Tycho. Bidrag till en monografi öfver Sveriges Sphaeropsideer. I. Sphaeropsideae et Melanconiceae novae in Suecia collectae. Öfversigt af Kongl. Vetenskaps-Akademiens Förhandlingar 54: 35-46. 1897.

⁶ Dearness, John. New and noteworthy fungi.—III. Mycologia 16: 143-176. 1924.

⁷ Karsten, P. A. Fragmenta mycologia XXIII. Hedwigia 27: 258-260. 1888.

¹ Published as Technical Paper No. 482 of the Oregon Agricultural Experiment Station, with the approval of the Director of the Station and the Chief of the Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

² Kienholz, J. R., and Leroy Childs. Fungicides in relation to scab and fruit russet of pear in the Hood River Valley, Oregon. Phytopath. 35: 714-722. 1945.

The experimental spray treatments, applied from the pink through 3 or 4 cover sprays during the 4 growing seasons, were as follows: (1) Check. Lead arsenate (3-100) only. (2) Micronized wettable sulphur (8-100) plus lead arsenate (3-100). (3) Ferric dimethyldithiocarbamate (Fermate) (1½-100) plus lead arsenate (3-100). (4) Copper phosphate-lime-bentonite (4-4-4-100) plus lead arsenate (3-100).

All materials were applied with a commercial power sprayer which maintained a pump pressure of approximately 400 pounds. The lead arsenate (3-100) for codling moth control usually was added in the calyx and later cover sprays.

Results. Four applications (pink, calyx, and 2 later applications) were made to the plots in 1942. Treatments 1 and 3 were each applied to 6 trees; treatment 2 to 60 trees; and treatment 4 to 24 trees. The design of this experiment did not permit a statistical analysis of the results. No counts were made of the number of blossoms originally on the trees, so the percentage that set fruit could not be determined. The average yields in field boxes per tree (a field box usually holds from 100 to 150 average sized pears and

TABLE 1.—*Effects of 3 fungicides upon the set of fruit of Anjou pears*

Treatment number and fungicide used	Percentage of blossoms setting and maturing their fruits		
	1943	1944	1945
1. Check trees	0.27	3.4	2.84
2. Wettable sulphur 8-100	0.11	1.9 ^a	1.80 ^a
3. Fermate 1.5-100	0.17	2.5	3.04
4. Copper phosphate-lime-bentonite 4-4-4-100	0.25	3.5	3.71 ^a

^a Results differ significantly from those of corresponding check at the 5 per cent point.

weighs approximately 45 to 50 pounds) were: Check, 9.8; wettable-sulphur, 3.3; Fermate, 8.0; and copper phosphate-lime-bentonite, 10.5. The sulphur-sprayed trees yielded about one-third as much fruit as the other trees.

Three applications (pink, calyx, and first cover) were made on the test plots in 1943. An improved experimental design involving the use of 3 replicates of 2-tree plots for each treatment was used. The blossoms were counted on each tree and the percentage of fruit set determined from the number of matured pears. Spring frost injury and other unfavorable weather conditions during the pollination period caused an extremely light set of fruit. The sulphur-sprayed trees had the lowest percentage of fruit set (Table 1), but the results were not statistically significant. The actual box yields are not available.

The plots received only 3 applications (pink, calyx, and first cover) in 1944. The experimental design was modified to provide 6 replicates of single-tree plots. The blossoms were counted and the percentage of fruit set determined, as in 1943, from the number of pears on the trees at harvest time (Table 1).

The 2.5 to 3.5 per cent fruit set in treatments 1, 3, and 4 produced a rela-

tively heavy yield per tree, but the 1.9 per cent fruit set in treatment 2 (sprayed with sulphur) produced only a medium crop. The lesser fruit set in treatment 2 was statistically significant by odds of 19:1. The average yields, in field boxes per tree were: Check, 13.2; wettable-sulphur, 9.2; Fermate, 11.4; and copper phosphate 14.4.

Since the results in 1942, 1943, and 1944 indicated that the use of wettable-sulphur might cause serious reduction in yield, an enlarged experiment was designed in 1945. In this experiment 12 replicates of single-tree plots were distributed at random in a 16-year-old orchard. All the trees in this orchard had received uniform care for several years, and they produced a uniform and well-distributed crop in 1945, indicating that the selected site was ideal for an experiment dealing with fruit set.

Three applications (pink, calyx, and first cover) were made on all treated plots in 1945. The blossoms were counted on one main leader branch of each tree. This branch was chosen on the south or southeast side of the tree and every effort was made to select uniform limbs. The fruit counts were made after it was certain further loss of fruit would not occur.

The approximately one-third smaller set of fruit (Table 1) on the "count limbs" of trees sprayed with wettable sulphur, as compared with the trees receiving Fermate or lead arsenate only, was statistically significant. The set of fruit on the trees receiving copper phosphate was slightly greater than that of the check trees, with odds of 19:1 for significance. The average yields, in field boxes, by trees of comparable size in the plots were: Check, 11; wettable sulphur, 7.7; Fermate, 9; and copper phosphate, 10.9.

Since one tree may form twice as many blossoms as another of equal size, tree size does not make a true basis for a comparison of yields. The number of fruits produced from a given blossom potential provides better evidence of tree productivity.

Summary. A significant reduction in the yield of Anjou pears has been demonstrated in the Hood River Valley, Oregon, following the use of wettable-sulphur spray for pear scab control. Since wettable sulphur also has given poor scab control and caused fruit russet, it appears to be very undesirable as a fungicide for Anjou pears under conditions prevailing in the Hood River district. The appearance of fruit, yield, and foliage color have all been superior where such substitutes as copper phosphate or Fermate have been used.—J. R. KÜENHOLZ, Associate Pathologist, Division of Fruit and Vegetable Crops and Diseases, and LEROY CHILDS, Superintendent, Hood River (Ore.) Branch Expt. Station.

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The following forms are suggested for use in making a bequest to the American Phytopathological Society of America or for adding a codicil to a will.

FORM OF BEQUEST

I Give and Bequeath the sum of \$ to the AMERICAN PHYTO-PATHOLOGICAL SOCIETY OF AMERICA, a corporation, organized not for profit under the laws of the District of Columbia, such sum to be used for the general purposes of said organization.

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1. This is a Codicil to the last Will, dated the day of 19, of

2. I Give and Bequeath the sum of \$ to the AMERICAN PHYTOPATHOLOGICAL SOCIETY OF AMERICA, a corporation, organized not for profit under the laws of the District of Columbia, such sum to be used for the general purposes of said organization.

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SEVERAL SPECIES OF PYTHIUM PECULIAR IN THEIR SEXUAL DEVELOPMENT

CHARLES DRECHSLER¹

(Accepted for publication May 28, 1946)

Of the 15 fungi which in 1930 I briefly described (17) as new species of *Pythium* 12 have since been set forth more fully through illustrations and supplementary discussion (20, 21, 22, 23, 25). Similar discussion, together with figures at magnifications ($\times 500$; $\times 1000$) for the most part uniform with those of previous papers, is herein supplied for 2 of the remaining species, *P. oligandrum* and *P. salpingophorum*. As certain of the features characteristic of these forms can perhaps be better understood if opportunity is afforded for ready comparison with congeneric species, attention is devoted herein also to the morphology and development of *P. verans* de Bary and of *P. undulatum* Petersen *sensu* Dissmann. Occasion is taken besides to amplify the earlier accounts of my *P. anandrum* and my *P. periplocum*, especially with respect to antagonistic relationships and oospore germination.

MORPHOLOGY AND DEVELOPMENT OF PYTHIUM OLIGANDRUM

Pythium oligandrum has been found in a wide variety of phanerogamic host plants over an extensive range of latitude in the eastern half of the United States. Its diagnosis was drawn from a culture derived from a diseased pea (*Pisum sativum* L.) root mainly because in my earlier experience I encountered the species in impressive quantity among numerous cultures isolated from underground parts of canning peas affected with root rot. Thus, it was recognized in more than 20 cultures prepared from separate individual plants collected in the course of a pea-root-rot survey made during the unusually cold wet spring of 1924 in Maryland, Delaware, and New Jersey (14). It was found present also in more than a dozen cultures isolated somewhat later in the same season from softened pea roots sent to me by workers in Pennsylvania, New York, and Connecticut; and subsequently was identified likewise in 4 cultures among a more numerous collection contributed by F. R. Jones as being representative of the fungi found in a pea-root-rot survey carried out that year in Wisconsin (30). That the species is not restricted to peas soon became evident from its frequent appearance among a large assortment of *Pythium* cultures obtained in 1924 from blackened rootlets of sweet-potato (*Ipomoea batatas* (L.) Lam.) slips taken by L. L. Harter (27) from large roots planted in hotbeds near Rosslyn, Va. Isolation of the fungus from several assortments of decaying bean (*Phaseolus vulgaris* L.) roots collected near Pompano, Fla., in March and April, 1926, gave testimony to its existence in the South. During the exceptionally wet period beginning in the middle of August, 1926, it was iso-

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lated repeatedly from tomato (*Lycopersicon esculentum* Mill.) roots collected near Rosslyn, Va., as well as from roots of the giant ragweed (*Ambrosia trifida* L.) and of the pale touch-me-not (*Impatiens pallida* Nutt.) gathered in Washington, D. C. It made its appearance with considerable frequency among the fungi found developing in maize-meal-agar plates planted with discolored sugar-beet (*Beta vulgaris* L.) roots collected in fields near East Lansing, Mich., and near Saginaw, Mich., late in June, 1927. In 1928 it was recognized in cultures isolated from discolored sweet-pea (*Lathyrus odoratus* L.) rootlets originating from Long Island, N. Y.; and also in a culture derived from a candytuft (*Iberis* sp.) root from Maine. Before its description in 1930 the fungus had been received from Florida a second time; the second accession coming in cultures isolated from diseased tomato seedlings. In 1939 its occurrence in another southern State was made evident through receipt of a culture which according to a letter from A. A. Dunlap had been isolated from a diseased wheat (*Triticum aestivum* L.) root gathered in the Panhandle region of Texas.

Very often, as has been set forth earlier (24), *Pythium oligandrum* is encountered in root rot and damping-off in association with congeneric species familiar as causal agents of these diseases; the frequency of such association and the behavior of the fungus in dual culture with congeneric forms giving reason to believe that the species occurs in diseased roots less as a primary parasite of the various host plants affected than as a secondary invader subsisting partly on mycelia of primary invaders and partly on host tissues freshly killed by these mycelia. Nevertheless the species now and then occurs under circumstances indicating that it may not be wholly lacking in pathogenicity to higher plants. Thus, a number of bean pods found affected with watery decay in a garden near Delaplane, Va., late in August, 1926, after 2 weeks of rainy weather, promptly yielded *P. oligandrum* unaccompanied by any other fungus likely to have caused the decay. Again, among 64 *Pythium* cultures derived from separate cucumber (*Cucumis sativus* L.) fruits found affected with watery rot in a wet field near Beltsville, Md., in August, 1938, one culture clearly belonged to *P. oligandrum*; 3 of the others being identified as *P. ultimum* Trow, and the remaining 60 as *P. Butleri* Subr. The discovery of *P. oligandrum*, unaccompanied by any other likely pathogenic organism, in a cucumber fruit was contrary to expectations as the fungus has never been isolated from watermelon (*Citrullus vulgaris* Schrad.) fruits affected with blossom-end rot, though watermelon fruits are spontaneously attacked in the field by the closely related *P. acanthicum* and *P. periplocum*, which usually fail to develop in green cucumbers when inoculated by incision. When the cucumber strain of *P. oligandrum* was inoculated by incision into watermelon fruits left attached on the vine, it caused a progressive brownish decay (Fig. 1, A, B) very similar to the natural blossom-end rot due to *P. acanthicum*. On inoculation into healthy green cucumbers it caused watery decay (Fig. 1, C) in most though not in all instances. Several other cultures of *P. oligandrum*

when inoculated into watermelons gave results (Fig. 1, D) very similar to those obtained with the cucumber strain. However, their inoculation into cucumbers brought about infection less frequently, and their rate of advance in the invasion of infected specimens was appreciably slower (Fig. 1, E). Failure of the species to attack watermelon fruits spontaneously in the field would seem attributable mainly to the slow germination of its oospores—a character plainly adverse to ready establishment of a foothold in the tissues of the flower scar (20, p. 383). The cucumber strain apparently has greater capacity for infecting cucumber fruits than most cultures referable to the species. Because of its relatively unambiguous pathogenicity this strain has been used in preparing some of the accompanying figures; the other illustrations of the species being made mainly from 2 strains isolated from separate specimens of diseased pea roots collected in June, 1924, near Hamburg, N. Y., and near Mount Morris, N. Y., respectively.

When a random assortment of separately isolated cultures belonging to *Pythium oligandrum* are grown under similar conditions side by side the differences that come to light with respect to such macroscopic features as rapidity of mycelial extension, luster, and cumulous variegation, are ordinarily more pronounced than the differences evident in a comparable assortment of cultures belonging to *P. acanthicum*. On maize meal agar the submerged mycelium of *P. oligandrum*, unlike that of *P. acanthicum*, is from the start clearly visible to the naked eye. If the agar contains some finely divided maize meal in suspension, an arachnoid aerial mycelium will usually develop during the first 2 days; and after 4 or 5 or 6 days the medium often turns yellowish and in places may take on a somewhat crustose appearance owing to the maturation of oospores in enormous numbers. Under microscopical examination the mycelium gives the general impression of being much more delicate than the mycelium, for example, of *P. ultimum* or of *P. debaryanum* Hesse. The greater delicateness here is attributable, as in *P. acanthicum* and *P. periplocum*, to extensive development of the finer ramifications, since the main axial filaments are not markedly narrower than in the coarse-looking damping-off species. The operation of the delicate branches in effecting parasitic attack on congeneric forms has been set forth earlier (24, 25).

Asexual reproduction may be conveniently brought about by cutting out sizable slabs from a thinly poured Lima-bean-agar plate culture permeated with young mycelium of the fungus, and transferring them to a shallow layer of sterile water in a sterilized Petri dish.² At temperatures between 15° and 18° C. the irrigated tracts of mycelium give rise here and there to subspherical enlargements filled with densely granular protoplasm. These enlargements occur in varied relationship to the parent hyphae. Several of them may sometimes be found clustered near the tip of a short branch

² In order that the water may spread out thinly and not collect in thick unmanageable pools, the floor of any Petri dish intended to be used for zoosporangial development or for oospore germination should be freed of all greasy film by scouring it thoroughly with an abrasive cleanser.

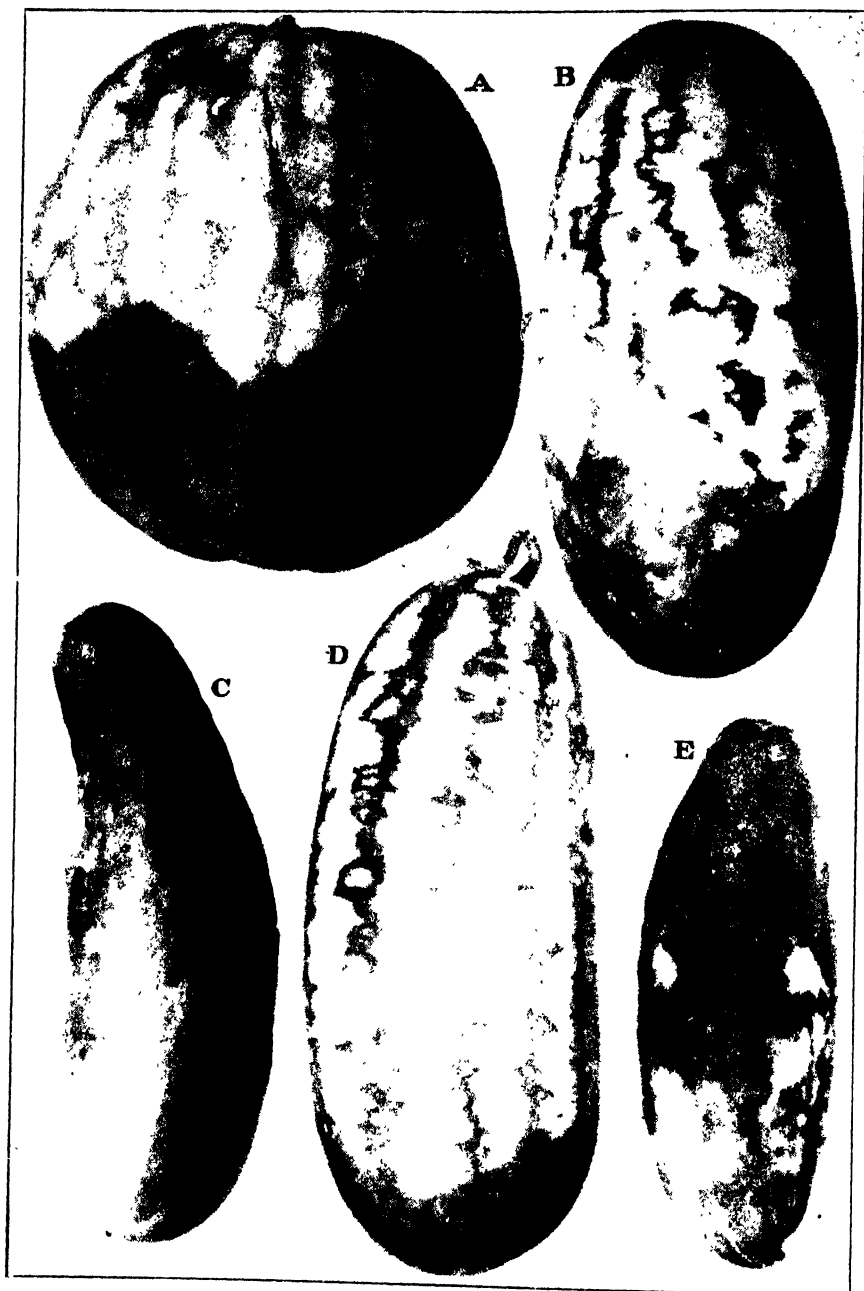


FIG. 1. Cucurbitaceous fruits attacked by *Pythium oligandrum*. A. Watermelon fruit 5 days after inoculation with the culture isolated from a Beltsville (Md.) cucumber; $\times \frac{7}{8}$. B. Watermelon fruit 9 days after inoculation with Beltsville cucumber strain; $\times \frac{7}{8}$. C. Cucumber fruit 5 days after inoculation with Beltsville cucumber strain; $\times \frac{1}{2}$. D. Watermelon fruit 9 days after inoculation with Hamburg (N. Y.) pea-root-rot strain; $\times \frac{5}{16}$. E. Cucumber fruit 9 days after inoculation with Hamburg pea-root-rot strain; $\times \frac{3}{8}$.

(Fig. 2, A), or again, may be compounded in a linear series which when delimited by cross-walls appears as a lobulate sporangial unit (Fig. 2, B). Often a sporangium may consist of a single globose enlargement together with an adjacent portion of hypha (Fig. 2, C, D), or of a conidium-like subspherical enlargement alone (Fig. 2, E, a), or of 2 or more globose enlargements together with connecting and adjoining hyphal parts (Fig. 2, E, b). Soon after it has been delimited the sporangial unit (Fig. 2, F) puts forth an evacuation tube (Fig. 2, F, t) which on attaining definitive length yields at the tip to permit migration of the protoplasmic contents into a terminal vesicle (Fig. 2, G) that often is only faintly discernible. When its migration is completed, the mass of granular material undergoes transformation into laterally biciliate zoospores after the manner usual in the genus *Pythium*: the motile spores (Fig. 2, H) being fashioned in the course of 15 to 25 minutes, and then escaping on disintegration of the vesicular film.

If left undisturbed the moderately thick wall of the evacuated sporangium (Fig. 2, G-W) retains its shape for some time, as does also the membrane of the evacuation tube (Fig. 2, G-W: t). The general make-up of the empty sporangial unit offers parallelism especially with *Pythium acanthicum*. In instances where the sporangium consists of a single globose part together with a relatively short cylindrical portion of the parent hypha, the evacuation tube more often arises from the cylindrical part (Fig. 2, F, G, I, J, L) than from the subspherical component (Fig. 2, K, M). Similar preference is evident likewise where the swollen component is bilobed (Fig. 2, H, P), or where the hyphal part is of considerable volume (Fig. 2, N), or where 2 globose parts are included (Fig. 2, Q-T: a, b). Occasionally 4 (Fig. 2, U, a-d; V, a-d) or 5 (Fig. 2, W, a-e) subspherical enlargements are found united into sporangial units somewhat more complex than can ordinarily be found in *P. acanthicum*; the evacuation tubes of such voluminous units arising sometimes from an expanded part (Fig. 2, U, t) and sometimes from a hyphal component (Fig. 2, V-W: t). While the tubes put forth by small sporangia (Fig. 2, L, t; M, t) may measure only about 15 μ in length, those extended from the more massive sporangia may attain lengths of 200 or 225 μ (Fig. 2, P, t; T, t). The empty tubes as a rule terminate abruptly with little apical modification: they rarely widen out markedly near the tip, are usually not lipped at the mouth, and apparently never are reflexed.

Sexual reproduction starts earlier and proceeds more rapidly in *Pythium oligandrum* than in most congeneric forms. In maize-meal-agar plate cultures kept at temperatures between 25° and 30° C. oogonia often begin developing abundantly within 30 hours after inoculation. They arise as globose hyphal enlargements in subterminal (Fig. 3, A) or intercalary positions (Fig. 3, B-D). Their growth is often directed toward one side with the result that their attachment may become more or less lateral (Fig. 3, E-I). During the later stages of expansion the individual oogonium puts forth protuberances from all portions of its surface. These protuberances

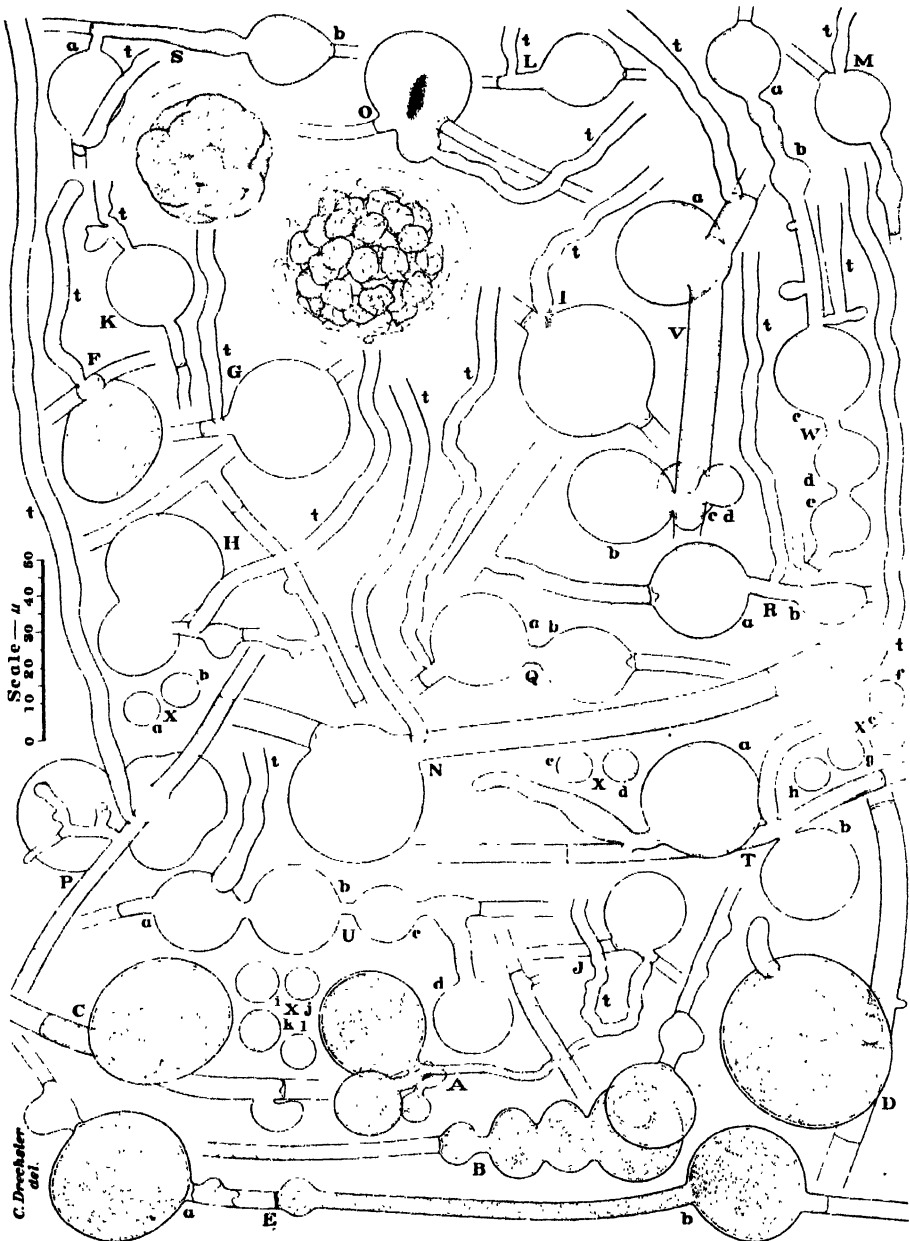


FIG. 2. Asexual reproduction in *Pythium oligandrum*; drawn to a uniform magnification with the aid of a camera lucida, from irrigated Lima-bean-agar preparations; all parts drawn from the Hamburg culture except K, L, M, S, and W, which were drawn from the Eden (N. Y.) pea root-rot strain; $\times 500$ throughout. A. Globose enlargement developing terminally on a hyphal branch. B-D; E, a, b. Sporangial units delimited by septa. F-H. Sporangia showing successive steps in zoospore production. I-P. Empty sporangia, each with a single expanded component. Q-T. Empty sporangia, each with 2 subspherical components, a and b. U, V. Empty sporangia, each with 4 globose components, a-d. W. Empty sporangium with 5 globose components, a-e. X. Encysted zoospores, a-l, showing variations in size and shape. (t, evacuation tube.)

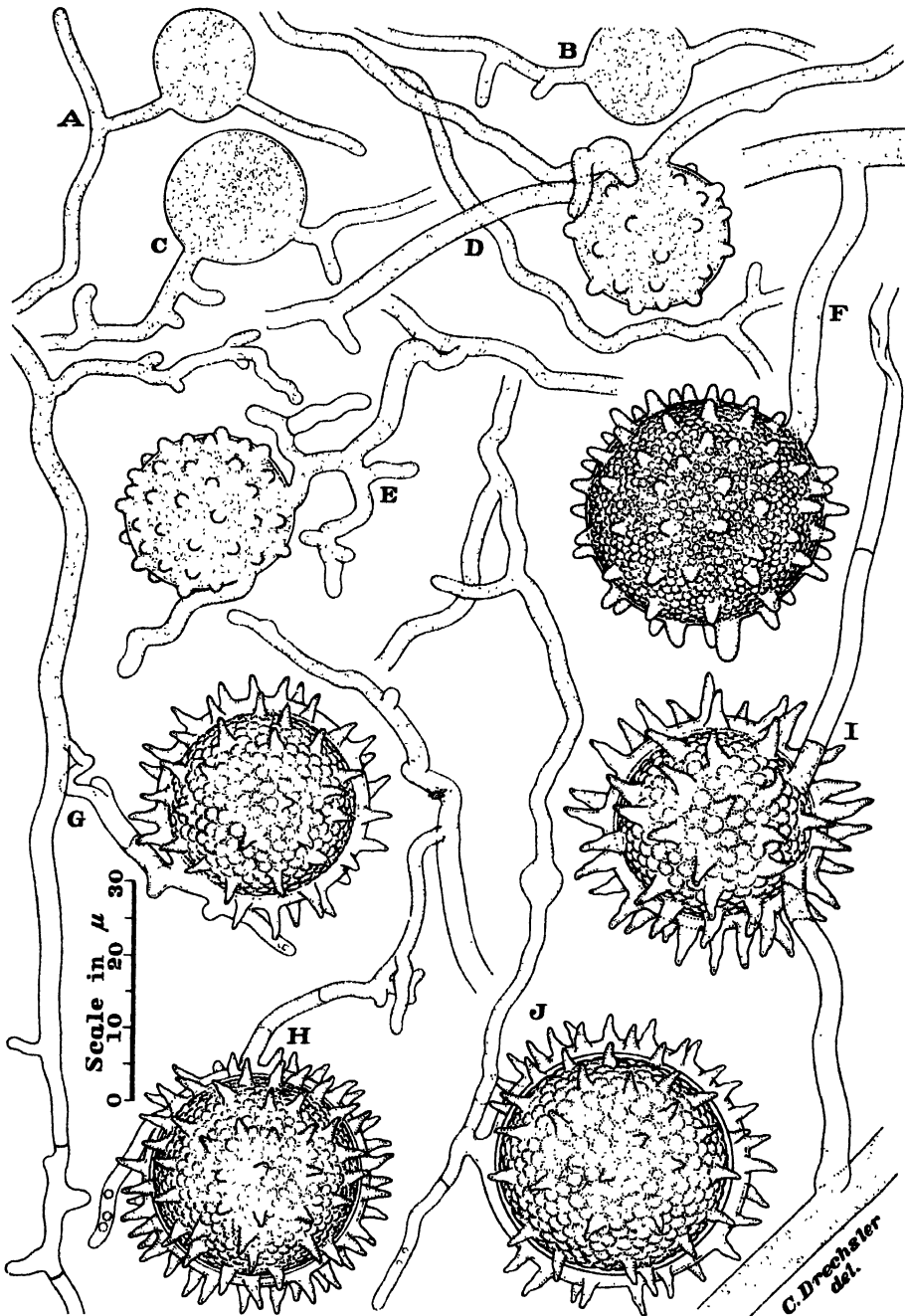


FIG. 3. Sexual reproductive apparatus of *Pythium oligandrum* (Hamburg strain); drawn with the aid of a camera lucida from maize-meal-agar cultures; $\times 1000$ throughout. A-C. Young growing oögonia. D. Nearly full-grown oögonium supplied with a young antheridium. E. Nearly full-grown oögonium. F. Full-grown oögonium shortly before deposition of basal septum; its contents consisting of small lumps. G-J. Parthenogenetic units in stage shortly before thickening of oospore wall.

are at first of a rounded, wartlike conformation (Fig. 3, D, E), but through apical elongation they soon acquire an irregularly conical, tapering shape. About at the time the spines are attaining their definitive length, the protoplasmic contents of the oogonium change from a densely granular to a coarse lumpy texture (Fig. 3, F). Soon afterwards the oogonium becomes delimited by deposition of 1 (Fig. 3, G, H) or 2 (Fig. 3, I, J) partitions, and its contents shrink away from its spiny envelope as the protoplasmic lumps composing them increase rather markedly in size. At this stage, wherever an antheridium (Fig. 4, A) or possibly 2 antheridia (Fig. 4, B) are present—application and development of a male complement takes place usually while the oogonial spines are being formed—fertilization is accomplished much as in the generality of related species. In any case, whether an antheridium is present (Fig. 4, C) or not (Fig. 4, E), the spherical protoplast soon secretes a thick wall, a homogeneous reserve globule gradually collects in the center, and the large protoplasmic lumps disintegrate to furnish material for the finely granular parietal layer through which plural refringent bodies of comparatively small size become distributed. Manifestly the oosphere undergoes conversion into an oospore of distinctive internal structure equally well by parthenogenesis (Fig. 4, F, G; Fig. 5, A, a, b; B-I) as after fertilization (Fig. 4, D).

The prevalence of parthenogenesis in *Pythium oligandrum* varies considerably among different strains of the species, and is, besides, subject to great variation from environmental causes. In the original diagnosis it was indicated that approximately 4 out of 5 oogonia developed parthenogenetically—this being the proportion most usually found when the strain used as type (one isolated from a discolored pea rootlet gathered near Eden, N. Y., in June, 1924) was grown on maize-meal agar at 24° C. Under like conditions the generally very similar Hamburg pea-root-rot strain (Fig. 3, 4, 5) shows virtually the same proportion of parthenogenetic reproductive apparatus. In the Beltsville cucumber-rot strain antheridia are commonly produced 2 or 3 times more abundantly (Fig. 6, A, B, C; Fig. 7, A-D), and as a result parthenogenesis occurs there in correspondingly lesser measure (Fig. 6, D-K). On the other hand, in the Mount Morris pea-root-rot strain 15 or 20 oogonia may often be found developing parthenogenetically (Fig. 8, A, B, D-Q) to every oogonium supplied with an antheridium (Fig. 7, E; Fig. 8, C). A striking illustration of variability from environmental causes was once provided by the Mount Morris strain when occasion was taken to compare a maize-meal-agar plate culture grown in the laboratory at about 27° C., with an irrigated Lima-bean-agar preparation stored in a refrigerator at 18° C. In the former scarcely one oogonium in a hundred was found supplied with an antheridium; whereas in the latter every oogonium was supplied with at least one antheridium, and many were supplied with two.

Whether formed in some abundance (Fig. 4, A-D; Fig. 6, A-C) or in relatively meager number (Fig. 8, C) the antheridia are mostly borne terminally on branches arising from a filament having no close mycelial

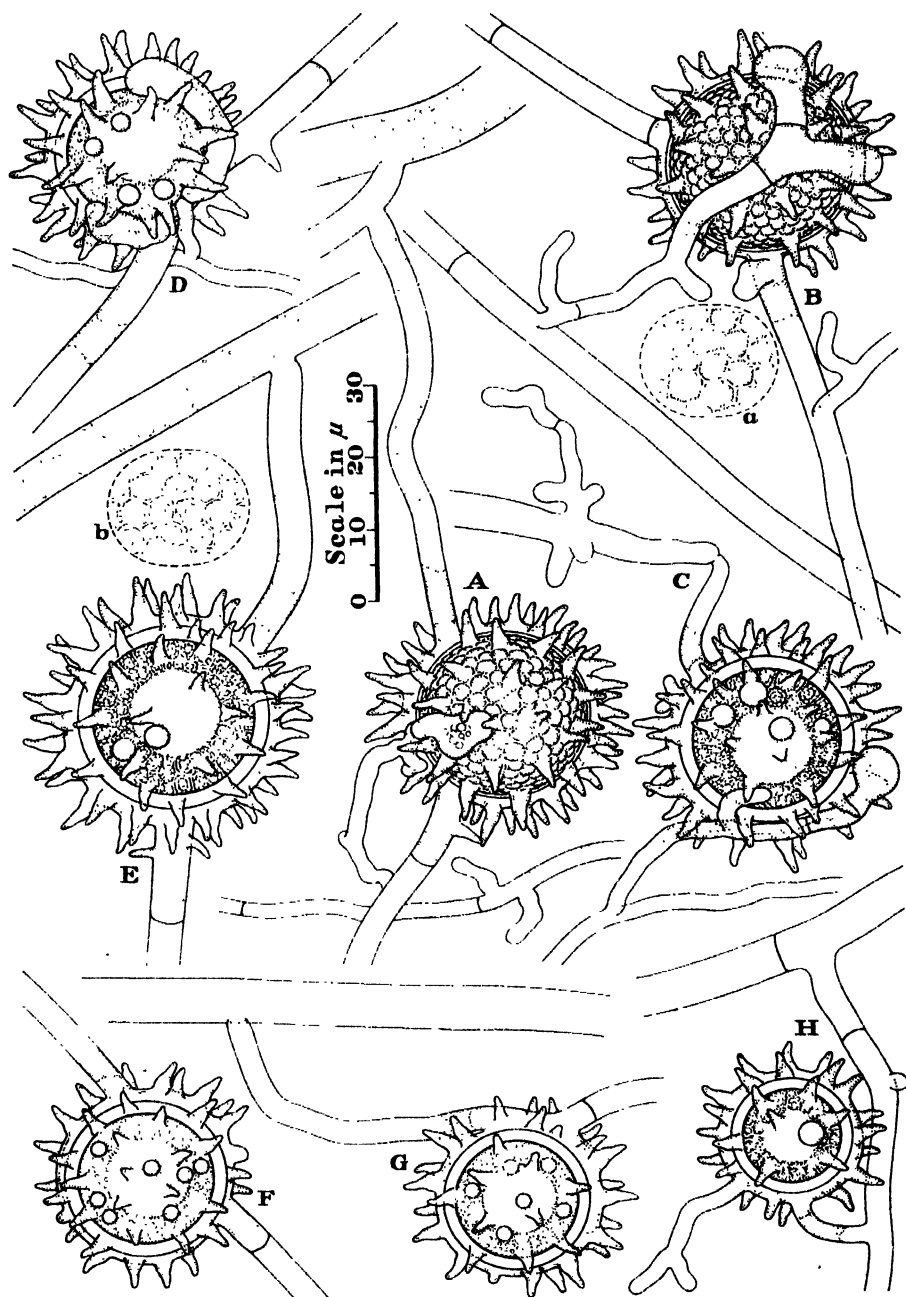


FIG. 4. Sexual reproductive apparatus of *Pythium oblongatum* (Hamburg strain); drawn with the aid of a camera lucida from maize-meal-agar plate cultures; $\times 1000$ throughout except in B, a, and E, b, where magnification is $\times 2000$. A-H. Six intercalary (A, B, D, E, F, G) and 2 terminal (C, H) oogonia, 4 among them (E-H) parthenogenetic, the others fertilized by 1 (A, C) or 2 (B, D) antheridia borne on a single branch arising from a neighboring hypha; the oospores in A and B in early lumpy stage, with lumps appearing in surface view as shown in B, a; oospores in C, E, H thick-walled, with fissured lumps appearing in surface aspect as shown in E, b; oospores D, F, G, fully mature.

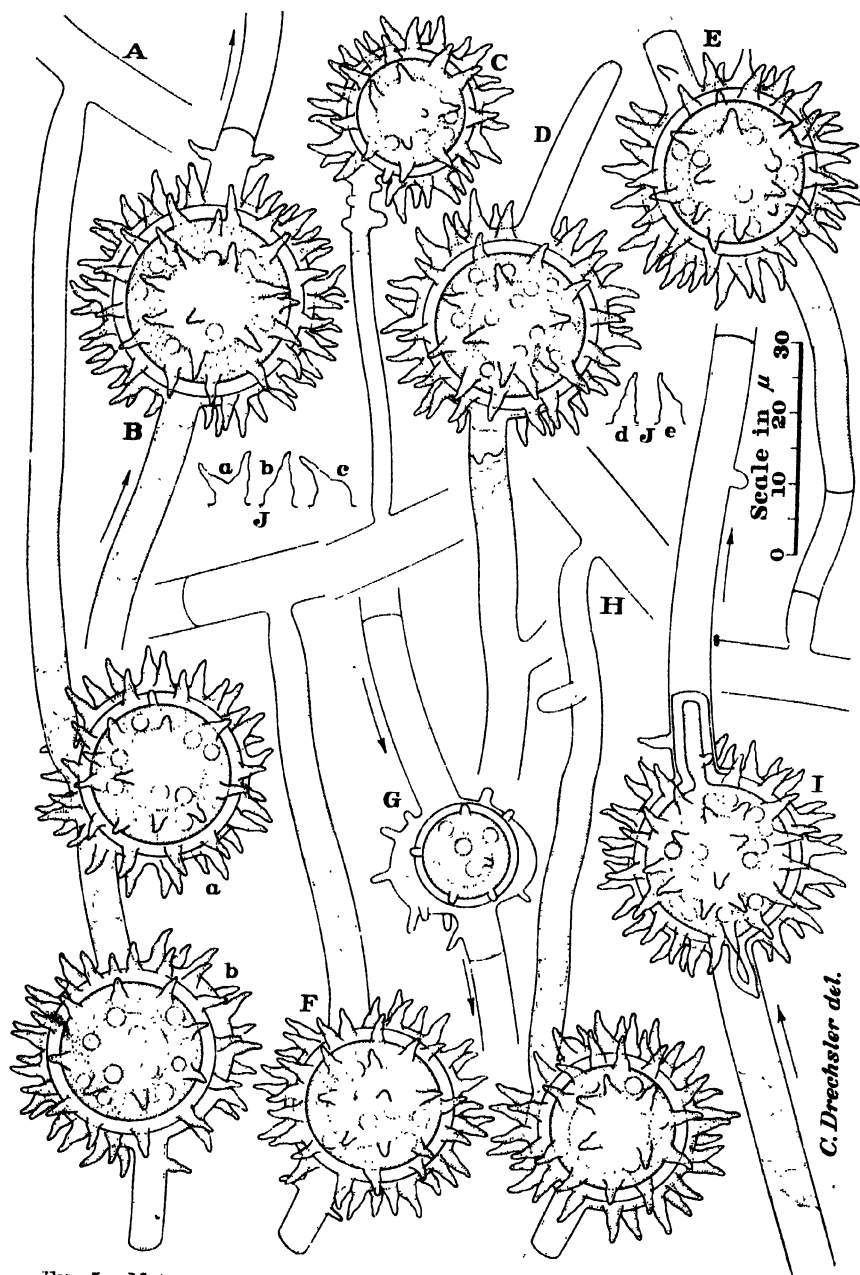


FIG. 5. Mature sexual reproductive apparatus of *Pythium oligandrum* (Hamburg strain); drawn with the aid of a camera lucida from maize meal agar plate cultures; $\times 1000$ throughout. A. Two adjacent intercalary oogonia, a and b, the proximal one delimited by a plug on both sides. B-I. Eight oogonia varying in size and shape, 6 among them (B, E-I) intercalary and 2 terminal (C, D) in position; G being an under-sized specimen beset with poorly developed protuberances and delimited by crosswalls both proximally and distally; the others (B-F, H, I) being delimited proximally by a plug which in some instances (B, D, F, H, I) is shrinking or disintegrating. J. Well-developed oogonial protuberances, a-c, showing irregularly tapering shape and somewhat blunt tip.

connection with the hyphal element bearing the oogonium. However, with some search in agar cultures rather soon after sexual reproduction has begun, and before too many filaments and branches have faded from view, instances can usually be found where the mycelial connection between antheridium and oogonium can be traced with certainty. The unit of sexual apparatus shown in figure 7, A, where the combined length of oogonial branch (Fig. 7, A, a), antheridial branch (Fig. 7, A, b), and intervening hyphal elements is approximately 225 μ , may be taken as illustrating an unusually close mycelial connection; though an even closer connection, where the total length of the communicating elements is only about 125 μ , is shown in figure 7, B. In most instances where a mycelial connection between the conjugating organs can be followed successfully amid the confusion of hyphae, the aggregate length of the communicating parts varies from 250 to 600 μ (Fig. 7, C-E).

As might be expected in view of their usually somewhat extensive application to oogonia rather closely beset with spines, the antheridia of *Pythium oligandrum* vary considerably in size and shape. Some of the smaller specimens, consisting merely of a slightly crook-necked inflated terminal segment (Fig. 4, A), are not greatly different from the sessile monoclinal antheridia familiar in *P. ultimum*. More often, however, the delimiting septum is laid down some distance backward from the expanded tip, so that the male cell comes to include a tubular portion tapering gradually toward the base (Fig. 4, C, D; Fig. 6, A, B). Frequently, owing to terminal branching or to local constriction, an antheridium may be distinctly lobate (Fig. 7, A, D). Where ramification of the antheridial hypha has led to the formation of 2 fairly massive branches, cross-walls may be laid down to delimit each as a separate antheridium (Fig. 4, B, D; Fig. 6, C).

The production of terminal branch antheridia by *Pythium oligandrum* might perhaps be held to distinguish this species adequately from *P. arlotrogon*, in which according to de Bary's (3, 4) original descriptive statements fertilization was seen to be accomplished by an antheridium consisting always of a hyphal segment adjacent to the oogonium. However, since in many cultures of my fungus branch antheridia are often only sparingly produced, and indeed are sometimes almost wholly lacking, the absence of such easily recognized male cells in de Bary's material could hardly be considered a fully decisive distinguishing feature should *P. oligandrum* be found to produce adjacent cylindrical antheridia as well as branch antheridia. Much material was examined, therefore, to determine whether in the species oogonia not supplied with branch antheridia develop parthenogenetically or at times, if not always, are fertilized by an adjacent male segment. Fertilization of the oogonium by passage of protoplasmic materials through the partition delimiting it at the proximal end should be especially subject to observation, for as a rule this partition is not an ordinary membranous cross-wall but a massive plug, usually 3 to 10 μ long, composed of a gelatinous substance (Fig. 3, G-I; Fig. 4, A-H; Fig. 5, A, C, E; Fig. 6, A-G, I;

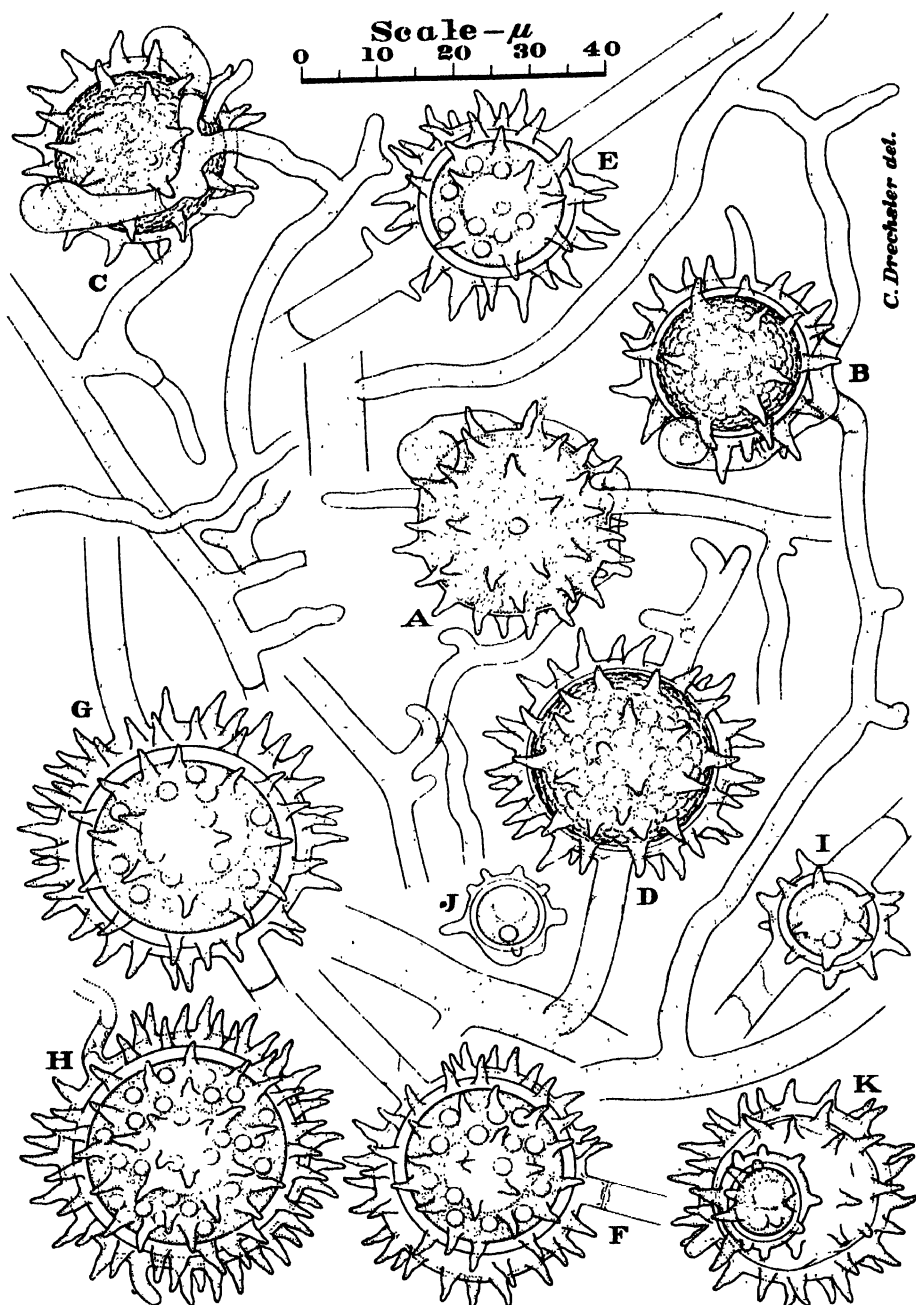


FIG. 6. Sexual reproductive apparatus of *Pythium oligandrum* (Beltsville cucumber strain); drawn from maize-meat-agar plate cultures with the aid of a camera lucida; $\times 1000$ throughout. A–H. Eight well-developed oogonia, each delimited proximally by a plug; two (A, B) being supplied with 1 antheridium, one (C) with 2 antheridia, the others (D–H) lacking antheridia; in A the contents are still granular, in C the lumpy oospore has contracted, in B and D the oospore wall is being deposited, in E–H the parthenospore is fully mature. I. Small intercalary oogonium with mature parthenospore showing only 2 refringent bodies. J. Very small terminal oogonium with a small parthenospore of unitary internal make-up. K. Oogonium whose envelope surrounds an oospore wall that encloses a very small secondary oogonium wherein is contained a mature parthenospore of unitary organization.

Fig. 7, A-D; Fig. 8, A, C, E) apparently similar to the substance secreted where hyphae are cut or wounded. This plug remains intact not only during the period in which fertilization might take place, but for many days after the oospore is fully mature, until eventually it undergoes gradual shrinkage and alveolar disintegration (Fig. 5, B, F, H, I; Fig. 6, II; Fig. 8, D). When 2 oogonia are formed adjacent to each other (Fig. 5, A, a, b) the proximal one (Fig. 5, A, a) is commonly bounded by a plug on each side; and occasionally a solitary oogonium (Fig. 8, A, F) is likewise delimited by plugs both proximally and distally. As passage of protoplasmic material through a plug never has come under observation, it can hardly be doubted that development of intercalary oogonia bounded by plugs at both ends and of terminal oogonia bounded proximally by a plug (Fig. 3, G, II; Fig. 4, C, H; Fig. 5, C, D; Fig. 6, D) must be parthenogenetic wherever a branch antheridium is lacking.

The commonplace membranous cross-walls usually delimiting intercalary oogonia at the distal end—such cross-walls are found bounding at both ends some intercalary oogonia (Fig. 5, G; Fig. 8, G) presumably formed after the mycelium had become too largely exhausted for the isolated living remnants to retain much polarity—offer greater difficulty in determining presence or absence of fertilization like that ascribed to *Pythium artotrogus*. Since in that species de Bary could see the empty fertilization tube only in favorable instances, it may be inferred that in most units of mature sexual apparatus the antheridial character of an adjacent hyphal segment was evidenced only by an aperture in the delimiting septum. Such an aperture, if present in *P. oligandrum*, would almost certainly be obscured beyond all recognition in the many instances where several oogonial spines are found projecting out in positions directly above and below the septum. Fortunately, in my fungus the distal delimiting wall is frequently placed well beyond the tips of all distally projecting spines, and thus can often be scrutinized to good effect. Yet in no instance has any likely aperture been discovered, nor has any tubular or funnel-shaped modification been seen that could be held to have derived from antheridial activity. Furthermore, oogonia with filamentous hyphal prolongations, often 25 to 50 μ long (Fig. 5, D, G, I; Fig. 8, D), were often taken under observation, especially during the contraction of the protoplast, to determine whether an antheridium might then be present, contributing its contents after complete dissolution of a temporary partition. So far no good evidence of such broad conjugation has been uncovered. Consequently the view that adjacent antheridia are wholly absent in *P. oligandrum*, and that wherever an oogonium is not supplied with a branch antheridium its development takes place parthenogenetically—a view incorporated into the diagnosis of the species—still seems well justified.

However the portion of the diagnosis setting forth the refringent body in the oospore as "often not clearly in evidence, when visible often sub-spherical 3 to 4.5 μ in diameter" has required emendation (26). The un-

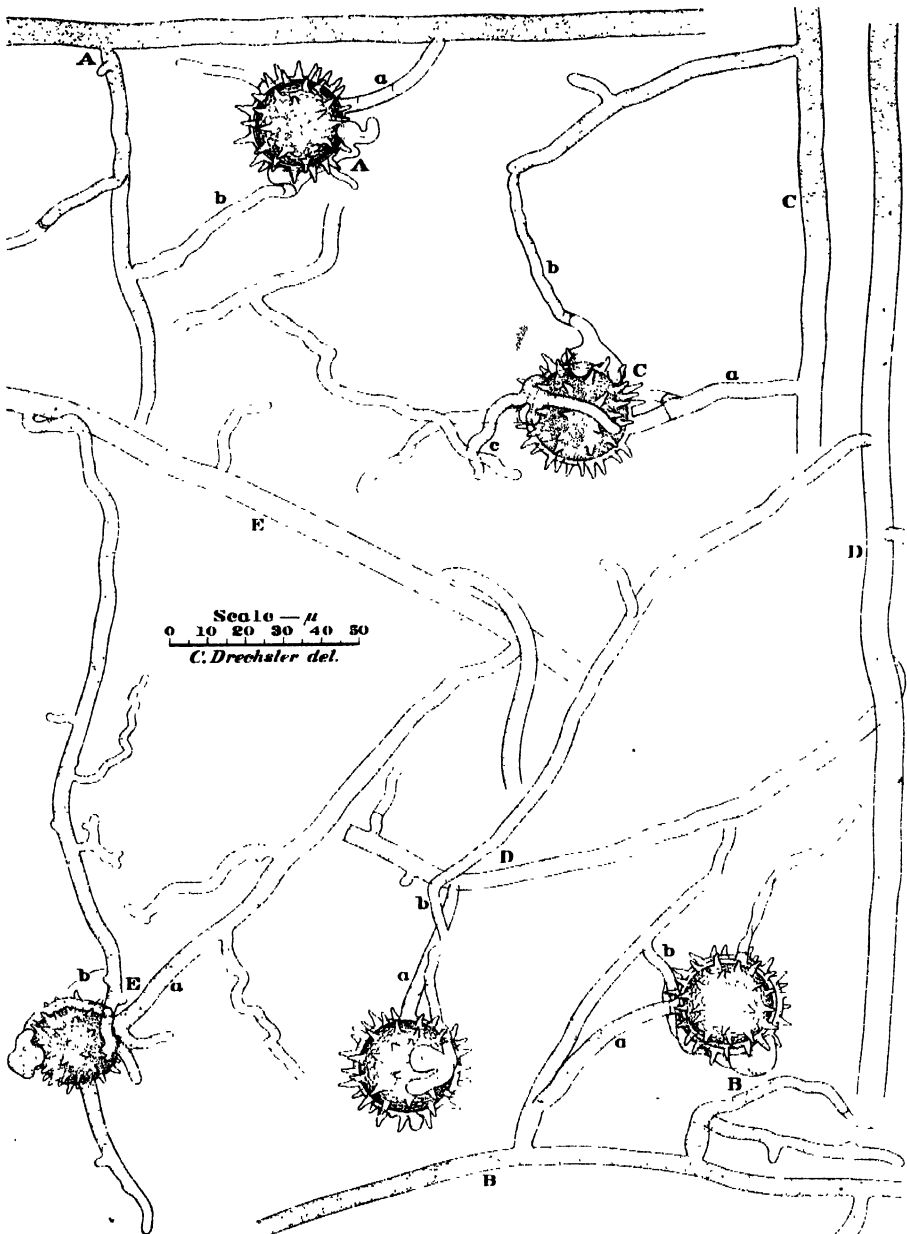


FIG. 7. Sexual reproductive apparatus of *Pythium oligandrum* showing mycelial connection between oogonium and antheridium; drawn from maize-meal-agar plate cultures at a uniform magnification with the aid of a camera lucida; $\times 500$ throughout. A, B. Units of sexual apparatus of the Beltsville cucumber strain, showing unusually close mycelial connection of the paired organs. C, D. Units of sexual apparatus of the Beltsville cucumber strain, showing the rather remote mycelial connection more usually found; in C an additional antheridium is evidently being supplied by a branch, e, from a neighboring hypha. E. Unit of sexual apparatus from Mount Morris (N. Y.) pea-root-rot strain; oogonium full grown but not yet delimited from supporting branch; showing the rather remote mycelial connection usually found. (a, oogonial branch; b, antheridial branch.)

happy phrasing of the passage cited came from perplexity as to what constituted correct internal organization in oospores of the species; the refringent bodies here being not especially hard to see, but assuredly difficult to recognize in their true character if the observer is strongly expectant of the unitary organization common to most pythiaceous fungi, including the very intimately related *Pythium acanthicum* and *P. periplocum*. At the early stage in development of the oospore, when in most congeneric forms the single refringent body first becomes visible among the protoplasmic lumps surrounding it, *P. oligandrum* likewise often shows only one such body. Indeed, sometimes in rather small oospores (Fig. 4, II) only a single refringent body may be present in the somewhat later stage when the protoplasmic lumps have begun to show the minute concentric and radial fissuring whereby they become resolved into minute granules (Fig. 4, E, b); though in most cases 2 to 4 of these cellular components are then revealed (Fig. 4, E, C'). As maturation proceeds the refringent bodies increase to their definitive number, which for oospores of usual dimensions, formed at temperatures between 25° and 30° C., varies commonly from 4 to 15 (Fig. 4, D, F, G; Fig. 5, A-I; Fig. 6, E-G; Fig. 8, D-F). In some exceptionally large oospores (Fig. 6, II) as many as 25, 26, or 27 refringent bodies have been counted, while some decidedly small specimens (Fig. 6, I; Fig. 8, G, H) have shown only two. After diligent search very small oospores have even been found that at maturity contained only a single refringent body and thus displayed the unitary organization familiar in related fungi. These minute spores, only about 10 μ in diameter, were apparently formed late from small isolated remnants of living mycelium (Fig. 6, J), or occasionally were produced in a secondary sporangium within primary reproductive apparatus whose contents for the most part had suffered degeneration (Fig. 6, K).

Although the multiplication of the refringent bodies entails reduction of their diameter to about 2 or 2.5 μ , they remain rather conspicuous in the finely granular parietal layer of the mature spore. Once the unusual association of plural refringent bodies with a single reserve globule has become familiar as a distinctive feature of the species, *Pythium oligandrum* can be recognized solely by its resting oospores; its identification then being possible even in old isolation cultures, heavily contaminated with bacteria, where all membranous vestiges of hyphae, antheridia, and spiny oogonia have long disappeared from view. Although old oospores of congeneric forms often show multiple spherical vacuoles in the parietal layer, these vacuoles can usually be distinguished from the plural refringent bodies of *P. oligandrum* by their larger and frequently more variable size. Larger size likewise pertains to the 2, 3, or 4 refringent bodies found in oospores of some congeneric species after their unitary structure has undergone modification through prolonged aging. The multiplication of refringent bodies in *P. oligandrum* cannot similarly be ascribed to after-ripening, as it is usually accomplished in maize-meal-agar throughout the expanse of a Petri-plate culture 100 mm. in diameter within 5 or 6 days after inoculation—at a time

when in parallel cultures *P. ultimum* and *P. debaryanum* have often not yet begun to produce sexual apparatus.

Among the oogonia that in addition to a subspherical spiny part include a rather extensive filamentous prolongation at one end or at both ends, some fail to collect their contents wholly within the subspherical part; so that the oospores will bear a projection, often more or less cylindrical, at one or at both poles (Fig. 5, I). Under certain conditions of development many oogonia may be formed consisting of a relatively small globose part together with much more voluminous prolongations, which sometimes are smooth (Fig. 8, I, J) and sometimes are elaborately beset with spiny protuberances (Fig. 8, K). Such rangy oogonia commonly produce an elongated oospore whose irregularly cylindrical shape is modified by a bulbous enlargement (Fig. 8, I; J, a; K, a), and frequently, in addition, give rise to a second cylindrical oospore having no bulbous modification (Fig. 8, J, b; K, b). Occasionally hyphal segments, wholly devoid of outward differentiation, function as oogonia in giving rise endogenously to elongated cylindrical oospores (Fig. 8, L-Q), or in extreme instances to filamentous oospores 100 to 300 μ long and 3 to 4 μ wide. The reserve material in cylindrical and filamentous oospores is divided among a variable number of globules which like the plural refringent bodies are arranged longitudinally at moderate intervals (Fig. 8, I-P). Obviously the organization here imposed by spatial necessities is not equal in descriptive merit to the multiply organization characteristic of the ordinary subspherical oospores of my *Pythium helicoides* and its close allies. Some cylindrical oospores reveal plural reserve globules and a single refringent body (Fig. 8, K, b; Q), thus reversing the normal numerical relationship of these cellular components.

The metrical data given in the original diagnosis relative to size of oogonium and oospore were based on 200 measurements of the Eden pea-root-rot strain grown in maize-meal-agar plate cultures under the same conditions as the cultures of *Pythium acanthicum* and *P. periplocum* utilized for measurements previously reported (20, p. 402, 406). The 200 oogonia chosen at random gave values for diameter, expressed in the nearest integral number of microns, distributable as follows: 17 μ , 2; 19 μ , 2; 20 μ , 1; 21 μ , 1; 22 μ , 3; 23 μ , 8; 24 μ , 23; 25 μ , 27; 26 μ , 31; 27 μ , 36; 28 μ , 31; 29 μ , 14; 30 μ , 11; 31 μ , 5; 32 μ , 2; 33 μ , 2; 35 μ , 1. And the oospores, all of correct internal organization, that were contained in these oogonia, gave values for diameter distributed as follows: 15 μ , 2; 17 μ , 2; 18 μ , 1; 19 μ , 2; 20 μ , 8; 21 μ , 24; 22 μ , 33; 23 μ , 44; 24 μ , 36; 25 μ , 22; 26 μ , 15; 27 μ , 6; 28 μ , 3; 29 μ , 1; 30 μ , 1. While the 3 closely related echinulate species are thus rather similar in their main dimensions, the generally smaller size of *P. acanthicum* is nearly always directly recognizable when microscopical comparison is made between representative cultures grown under similar conditions or between assortments of such cultures. In such comparison *P. periplocum* is not found consistently smaller than *P. oligandrum*, since its oogonia and oospores are virtually of the same size as those of numerous strains of the latter species,

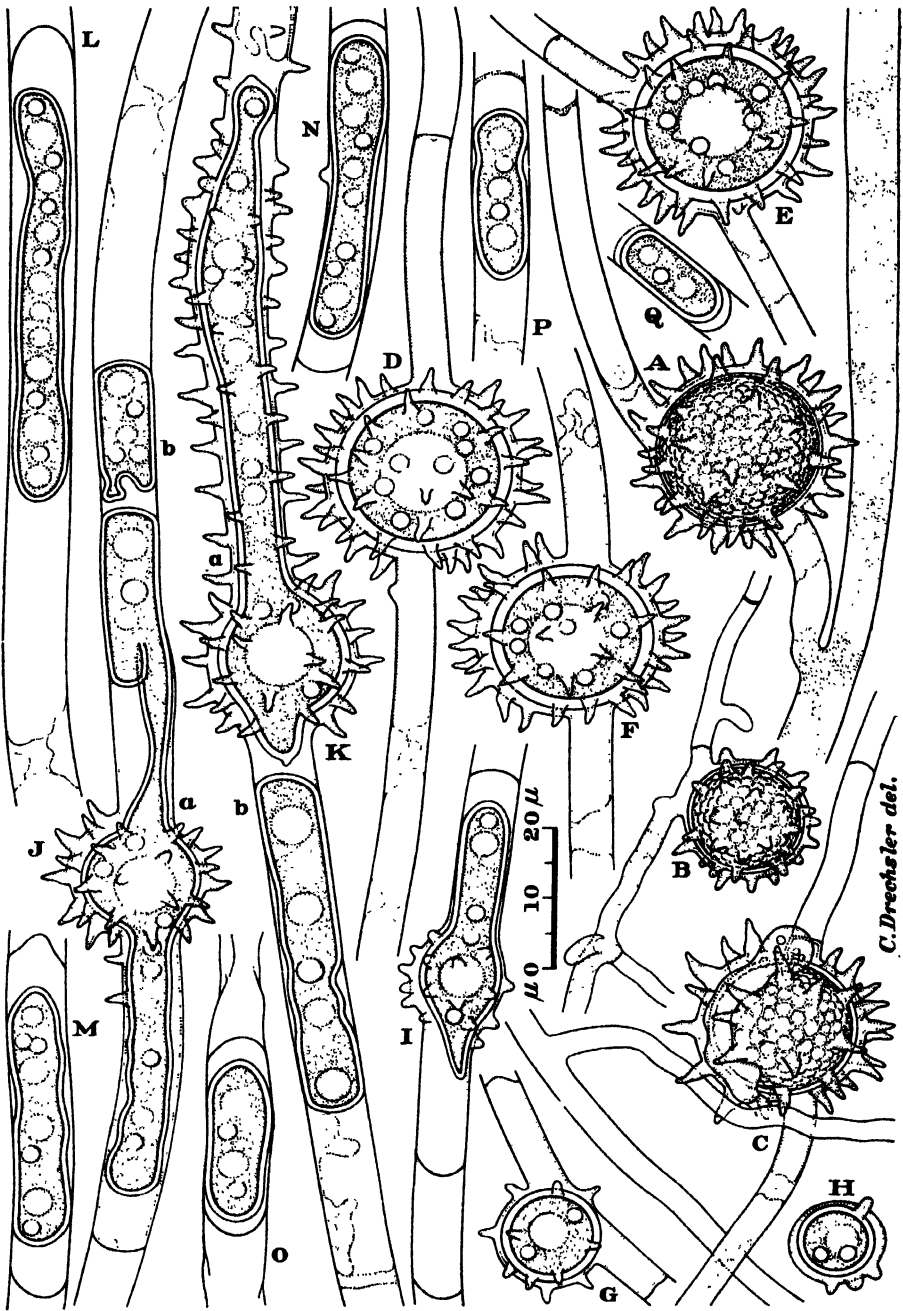


FIG. 8. Sexual reproductive apparatus of *Pythium oligandrum* (Mount Morris pea-root-rot strain) drawn from maize meal-agar plate cultures with a camera lucida; $\times 1000$ throughout. A, B. Intercalary parthenogenetic oogonia, each with a parthenospore in early stage of development. C. Intercalary oogonium supplied with an antheridium. D-F. Intercalary oogonia of ordinary size, each with a mature parthenospore. G, H. Small oogonia, each with a small parthenospore that contains only 2 refringent bodies. I-Q. Oogonia of aberrant shapes, each with a mature parthenospore, except J and K, which contains 2 parthenospores, a and b.

including, for example, the Mount Morris strain. Nevertheless, owing to the larger dimensions of many other of its strains, such as the Hamburg pea-root-rot strain and the Beltsville cucumber-rot strain, *P. oligandrum* may rightly be considered the somewhat more robust species. Its oogonial protuberances (Fig. 5, J, a-e), on the whole, appear longer, more irregularly contoured, more pronouncedly tapered, and more numerous than those of *P. acanthicum* and *P. periplocum*.

Under natural conditions oospores of the soil-inhabiting Pythiaceae presumably germinate for the most part in water containing no nutrient materials either in solution or in suspension. When bathed in sterilized distilled water newly matured oospores of *Pythium oligandrum* give no sign of germination. After resting for 40 or 50 days a small proportion of oospores will usually germinate when immersed in a shallow layer of water. Practically all oospores in maize-meal-agar culture become capable of germination after aging for 150 to 200 days. Onset of germinative development is manifested by change of the reserve globule from a spherical to an irregular shape. The refringent bodies gradually become less distinctly visible, while at the same time radial markings appear in the oospore wall, or rather in the somewhat darker inner layer making up about two-thirds of the thickness of this wall (Fig. 9, A). Soon the refringent bodies are wholly lost to view, and the radially striate darkish layer merges indistinguishably with the granular protoplasmic mass, which thereby comes to extend to the persistent thin clear outer layer of the oospore wall (Fig. 9, B). The spherical cell, now thin-walled, buds forth a protrusion that on penetrating the oogonial envelope continues growth outside as a germ hypha (Fig. 9, B, t; C, t). On abrupt yielding of the hyphal tip (Fig. 9, D, t) the protoplasmic contents flow into a terminal vesicle to be fashioned into laterally biciliate zoospores; the number of the spores produced varying usually from 5 to 15. The empty evacuation tube here commonly ranges from 10 to 50 μ in length, and from 3 to 4.5 μ in greatest width (Fig. 9, E-L, t). In most instances it terminates abruptly without distal modification, though occasionally its mouth is minutely lipped (Fig. 9, D, t; I, t). Most frequently the evacuation tube is found coming out directly from the spherical envelope of the oogonium, yet now and then it erupts from a filamentous prolongation (Fig. 9, I, t). Wherever germ hyphae attain lengths much in excess of 50 μ without functioning as evacuation tubes, and begin to ramify (Fig. 9, M), zoosporangial reproduction has obviously been abandoned in favor of mycelial growth. Instances of such abandonment are not frequent if the water layer is shallow and is left undisturbed; so that in carefully managed preparations well after-ripened oospores will begin to liberate zoospores within 4 hours, and for about 6 or 8 hours longer will continue producing additional motile spores to provide often a far livelier display of active swimmers than can be obtained by irrigation of young mycelium. After a period of motility the zoospores round up (Fig. 9, N-X) much like those (Fig. 2, X, a-l) produced from sporangia of asexual origin. Later, as a

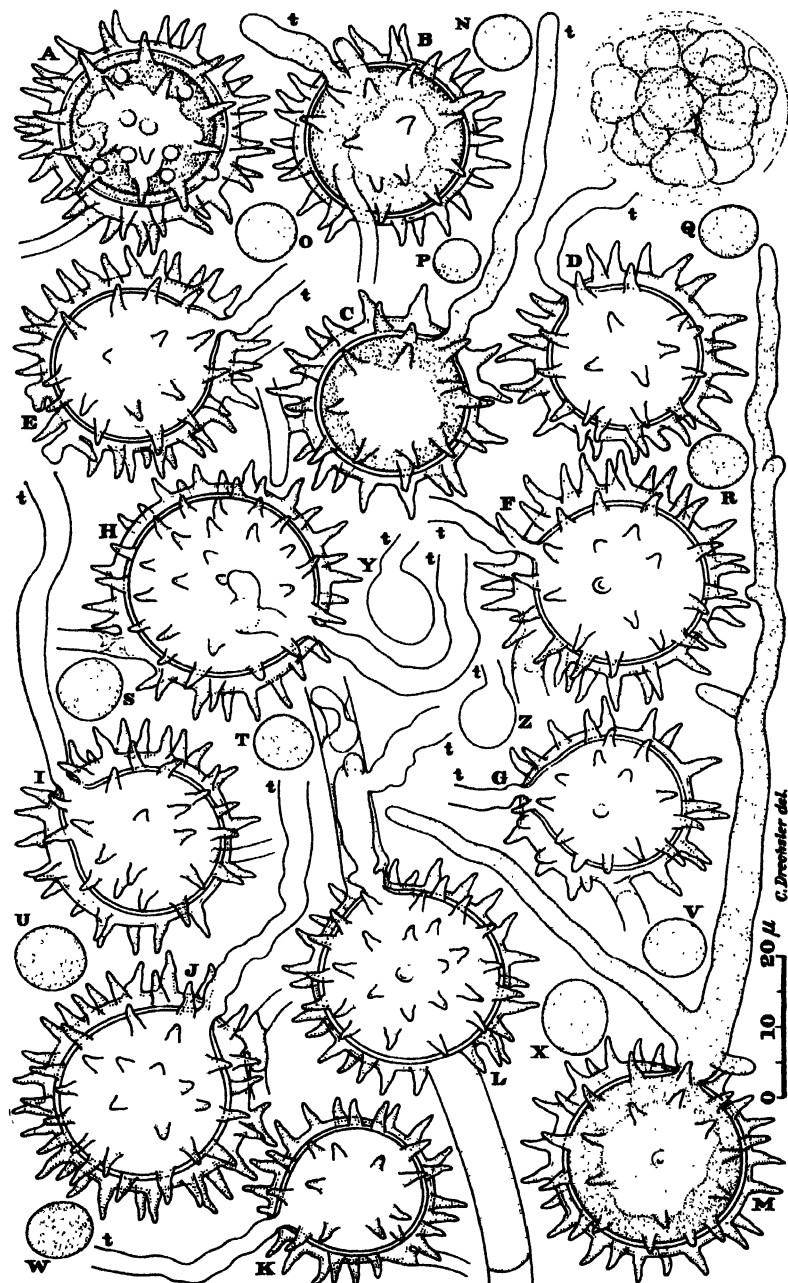


FIG. 9. Germination of oospores and parthenospores of *Pythium oligandrum* (Hamburg pea-root-rot strain) from maize-meal-agar plate cultures 6 months old; drawn with the aid of a camera lucida; $\times 1000$ throughout. A-D. Oospores showing, respectively, (A) assimilation of thick inner layer of wall by protoplast, (B, C) extension of germ hyphae, (D) individualization of zoospores in a vesicle formed terminally on the germ hypha. E-L. Empty membranous envelopes left after escape of swarmer from vesicle. M. Oospore germinating by production of a branching mycelial hypha. N-X. Encysted zoospores. Y, Z. Empty cyst envelopes after escape of a secondary zoospore from each. (t, evacuation tube.)

rule, they germinate vegetatively, though some few individuals usually develop iteratively, each discharging its contents through an evacuation tube (Fig. 9, Y, Z), 2 to 5 μ long and 2.5 μ wide, for conversion into a secondary laterally biciliate zoospore. The behavior of the fungus in the laboratory suggests that in nature it produces zoospores more abundantly from its germinating oospores and parthenospores than from zoosporangia formed by its mycelium.

Aside from the eastern United States, where my material of *Pythium oligandrum* originated, the species has been recorded from several other regions. Nattrass (36) in 1937 reported having isolated it 2 years earlier in Cyprus from immature fruits of *Prunus amygdalus* Batsch. Wager (42), who in 1931 cited "*Pythium* sp. cf. *P. artotrogus* (Mont.) de Bary" as having been found associated with wilting of a Shirley poppy (*Papaver rhoeas* L.) and of a snapdragon (*Antirrhinum majus* L.) in South Africa, subsequently (43) referred the fungus to *P. oligandrum*, at the same time making known that it had further been isolated in South Africa from wilting plants of marrow (*Cucurbita pepo* L.) and of cabbage (*Brassica oleracea* L. var. *capitata* L.). For the most part the descriptive particulars given by Wager agree with the morphology revealed in my cultures; yet it may be noted that one of his drawings (43: fig. 9, a) wherein an oogonium is shown fertilized by an antheridium borne terminally on a branch, about 37 μ long, arising from the oogonial stalk only about 7 μ from the female organ, pictures a closer mycelial connection between conjugating sex elements than has ever come under my observation in material of the species. The oogonial spines shown by Wager seem somewhat too sharply pointed at the tip, though they are otherwise of shape and stature usual in my cultures. Very sharply pointed spines are also shown in a figure of *P. oligandrum* recently published by Chesters and Hickman (12), who in England several years earlier (11) isolated from diseased roots of a cultivated violet (*Viola* sp.) some cultures held referable to the species. These authors depict an androgynous antheridium (12: fig. 3, D) that apparently has its origin even closer to the oogonium than the androgynous antheridium figured by Wager. No comment is made by Wager or by Chesters and Hickman concerning the presence of refringent bodies in the mature oospore, nor are such components recognizable in their illustrations. Middleton (34, p. 114, 115), who reported having found the species on the carrot (*Daucus carota* L.), the Christmas flower (*Euphorbia pulcherrima* Willd.), needlegrass (*Stipa* sp.), and wheat (*Triticum aestivum* L.) in the United States, described the oospore as containing a single reserve globule and refringent body.

During several decades preceding its description *Pythium oligandrum* could hardly have failed being encountered from time to time by investigators dealing with root rot and damping-off of the higher plants. It may be presumed that the usual disposition of the fungus in this earlier period is correctly suggested in Butler's report (42, p. 39) on the identity of the cultures isolated by Wager from snapdragon and Shirley poppy. Although

Butler failed to make out antheridia in these cultures and did not discover any evidence of hypogynal male cells such as he (10, p. 100) like de Bary (3, 4) before him had ascribed to *P. artotrogus*, and though he thought the cultures alien to the fungus he had figured in his monograph as well as to the fungus to which de Bary had originally applied the epithet, he nevertheless referred them "for the present in the collective species *artotrogus*." That the species *artotrogus* had in its application acquired a strongly collective character seems fairly certain. Most of the reports on its occurrence give little suggestion that details relating to antheridial morphology had received appropriate attention when the determinations were made; agreement with respect to size of oogonium, presence of oogonial protuberances, and size of oospore being apparently deemed sufficient to establish identity as long as no other species embodying somewhat similar features had been described in the genus. If in accordance with usage so broad, *P. acanthicum* and *P. periplocum* should, like *P. oligandrum*, have happened to be recorded under the binomial *P. artotrogus*, the error would have concerned species that presumably are intimately related to the one with which they were confused—all being distinguished by capacity for attacking congeneric forms, and by the tapering shape of their oogonial protuberances. Much wider of the mark would have been the almost equally probable application of de Bary's binomial to *P. spinosum* Saw. (39) and *P. mamillatum* Meurs (33), which through their more typically digitate oogonial protuberances, their rather copious production of aerial mycelium, and their inability to parasitize congeneric forms, are at once estranged from the *oligandrum* series and brought into alignment with the familiar damping-off pathogens *P. irregulare* Buism. (9), *P. debaryanum*, and *P. ultimum*.

Since several species of *Pythium* with spiny oogonia mostly 18 to 27 μ in diameter are now known to exist it can no longer be considered wholly certain that the fungus found by de Bary in dead tissues of herbaceous plant parts and described by him mainly from cress-seedling (*Lepidium sativum* L.) cultures as *P. artotrogus* was the same as the fungus which produced in a sprouted potato tuber the pronouncedly echinulate reproductive bodies whereon Montagne (5, 6, 35) more than 30 years earlier had based the generic and specific characterizations of *Artotrogus hydnosporus*. Accordingly, disagreement with de Bary's description can no longer be held necessarily to imply separateness from Montagne's species. The spines figured by Montagne (6: fig. 29) seem much narrower, more acutely pointed and more thickly crowded than any oogonial protuberances I have ever observed in cultures of *Pythium*; though in such particulars allowance must be made for wide differences in habits of draughtsmanship. Failure to mention or to depict antheridia might perhaps be held especially suggestive either of the consistently parthenogenetic *P. anandrum* Drechsl. (17, 20) or of the frequently parthenogenetic *P. oligandrum*; but the oogonia of these 2 species rather markedly exceed in diameter the 1/50 mm. indicated for this dimension by Montagne. Actually Montagne's fungus might have been a form

well supplied with antheridia; for if the male elements of *P. acanthicum* and *P. periplocum*, for example, soon become indiscernible even in a transparent substratum and under a very good modern microscope with excellent illumination, it seems at least probable that similarly evanescent elements could have remained undetected in more or less opaque potato tissue under a microscope of the sort used a century ago. In view of the attending difficulties it is not surprising that de Bary (4, p. 576), who later examined authentic material of *A. hydnosporus* in a dry permanent mount, was not able to give much further information with respect to the mycelial relationships and possible antheridial supply of Montagne's spiny bodies. Although de Bary held as unquestionably identical with these spiny bodies some echinulate structures which he had found in potato (*Solanum tuberosum* L.) tubers affected with the late blight fungus, *Phytophthora infestans* (Mont.) de Bary, and which he subsequently (4, p. 576) recognized as oogonia of his *P. artotrogus* containing oospores in mature or maturing condition, it is worthy of note that one (2, fig. 1) of his three early figures illustrating the echinulate structures seems to show 4 cellular components corresponding remarkably well in size to the plural refringent bodies of *P. oligandrum* while another figure (2, fig. 3) seems to show 2 such components. Possibly the comment (4, p. 576) in his definitive account of *P. artotrogus* to the effect that his earlier description had not adequately set forth the constitution of the oospore contents may have been intended to disparage the accuracy of these figures in showing plurally the cellular components under discussion. He stated (4, p. 624) at all events that at full maturity the condition of the oospore in *P. artotrogus* was like the condition illustrated in a ripe oospore of his *P. megalacanthum*, or in a ripe oospore of his *P. proliferum*—both revealing unmistakably a single refringent body in the parietal layer surrounding the single reserve globule. Likewise in another treatise (3, p. 61), devoted more especially to sexual reproduction in the oomycetes, he included all species of *Pythium* therein described by him—and *P. artotrogus* was one of these species—among representatives of various genera whose ripe oospore he found to contain a single "heller Fleck" (refringent body) in the parietal granular layer surrounding the "Fettknäuel" (reserve globule). While these statements by de Bary must be held to establish unitary organization of the oospore as a specific character of *P. artotrogus*, his curious failure to supply in his two later publications any figure showing unitary organization in a ripe oospore of the species invites speculation whether some difficulty may not have intervened such as could have been occasioned by intrusion now and then of material referable to *P. oligandrum*. Although Butler (10, p. 100-101) gave little attention to internal organization of oospores, his characterization of the antheridia in *P. artotrogus* as being "always hypogynal," together with his first-hand delineation of consistently hypogynal antheridia presumably from material of a spiny form he found in Calcutta in decaying potato tubers affected with *Phytophthora infestans*, would seem to provide the only record rather un-

ambiguously setting forth an association of the host relationship of *A. hydnosporus* with the antheridial morphology characteristic of *P. artotrogus*. Indeed, Butler's account would seem to provide also about the only first-hand record, apart from de Bary's description of *P. artotrogus*, wherein spiny oogonia are set forth as being fertilized exclusively by hypogynal antheridia. In recent times, however, Matthews (32, p. 101-104) described as *P. echinulatum* a fungus with spiny oogonia usually fertilized by hypogynal antheridia, rarely by branch antheridia. Though held to be similar to *P. artotrogus* in general appearance and in size of oogonia and oospores—the oospores individually containing a single refringent body—the fungus was separated from that species because it produced numerous conidia and zoosporangia. From the description given of them, these conidia and sporangia resemble rather closely the homologous reproductive bodies of *P. debaryanum* as well as the conidia of *P. ultimum*, which species de Bary evidently included under the one named in his honor. The resemblance seems of some moment, since in all of de Bary's cress-seedling cultures *P. artotrogus* never occurred except in admixture with *P. debaryanum*; the spiny form, according to his account, appearing tardily in somewhat meager quantity after the smooth damping-off parasite had produced a fairly luxuriant mycelium and numerous reproductive bodies. Under these circumstances should the spiny fungus have produced asexual reproductive bodies indistinguishable from those of *P. debaryanum*—and in this connection the difficulty of distinguishing generally similar bodies when seen only in mixture, together with the inferior capabilities of the microscopes in use 65 or 70 years ago, needs to be considered—de Bary might not, he rather clearly intimates (4, p. 574, lines 43 to 49), have been able to refer them to *P. artotrogus*. If seen only in mixed cultures of the kind studied by de Bary, even the sporangia of *P. oligandrum* and of *P. acanthicum*, though differing more pronouncedly from those of *P. debaryanum* than the sporangia described and figured by Matthews, might perhaps not be distinguished successfully from the more numerous alien reproductive bodies present with them. Wherefore, indeed, in separating these 2 spiny forms, and for that matter also *P. periplocum*, from *P. artotrogus*, it was deemed advisable to rely almost wholly on differences relating to morphology of sexual reproductive apparatus.

GERMINATION OF OOSPORES OF PYTHIUM PERILOCUM

Oospores of *Pythium periplocum* produced in maize-meal-agar plate cultures seem, like those of *P. oligandrum*, to require a fairly prolonged resting period before they will germinate readily in pure water devoid of nutrient substances. After some cultures had been stored in the laboratory for 165 days at temperatures fluctuating mostly between 28° and 32° C., about a third of the oospores germinated promptly on shallow irrigation. When storage was continued 45 days longer at slightly lower temperatures, nearly all the oospores germinated, some of them undergoing transformation into zoosporangia so rapidly that zoospores began swimming about within 2 hours.

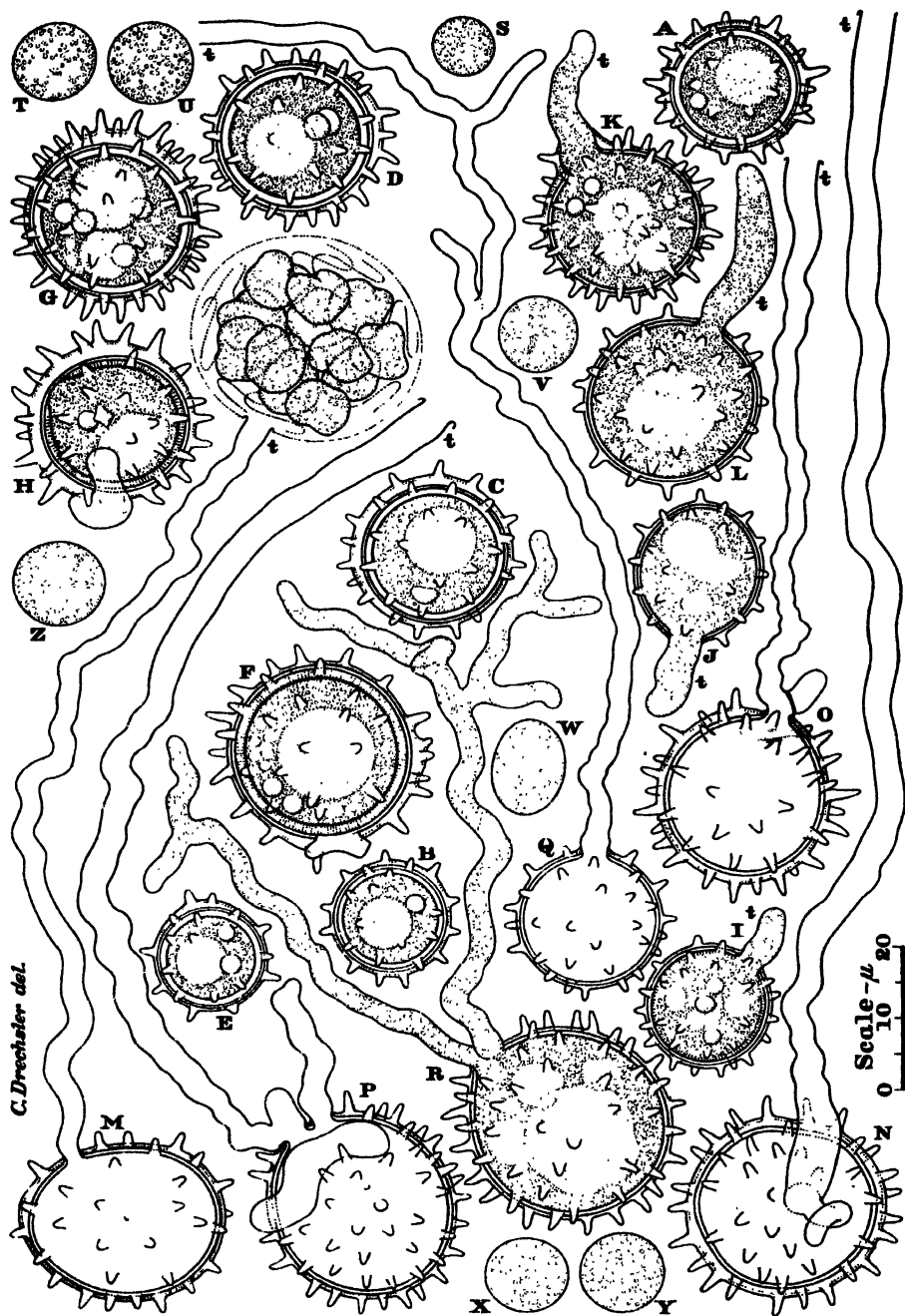


FIG. 10. Germination of oospores of *Pythium periplocum* taken from maize meal-agar plate cultures 7 months old; $\times 1000$ throughout. A-E. After-ripened oospores showing two layers in wall. F-H. Oospores about 30 minutes after transfer to water. I-L. Oospores with elongating germ hyphae, t. M. Oospore whose contents have been converted into zoospores in the vesicle at the tip of the evacuation tube t. N-Q. Empty oospore envelopes, each with an empty evacuation tube, t. R. Oospore germinating by production of a mycelium. S-Z. Encysted zoospores produced through germination of oospores.

During the resting period the oospore shows very gradually increasing contrast between a relatively thin colorless outer layer and a thicker yellowish inner layer (Fig. 10, A-G). In many instances the typically unitary organization of the protoplast (Fig. 10, A-C) becomes modified through the presence of 2 (Fig. 10, D, E, F) to 4 (Fig. 10, G) refringent bodies; the increased number of these bodies being often found associated with a noticeably vacuolate condition of the parietal granular layer (Fig. 10, F, G). Soon after an oospore is immersed in fresh water the thicker yellowish inner layer of its wall reveals innumerable closely arranged radial markings (Fig. 10, F). The striate layer now dissolves away in a localized region about 2.5 to 5 μ wide, permitting the protoplast to protrude against the thin outer layer (Fig. 10, H). Before long the outer layer yields in the region of contact as the protrusion presses forward into the oogonial chamber and then forces its way through the oogonial envelope to elongate externally as a germ tube (Fig. 10, I-L: t). In the meantime the striate inner layer of the oospore wall undergoes gradual obliteration throughout its circumference (Fig. 10, I), and soon merges indistinguishably with the protoplasmic mass, which thus is expanded to make contact everywhere with the persistent outer layer of the wall (Fig. 10, J-L); the reserve globule during the same period changing from a globose to a more irregular shape (Fig. 10, H, L), or often dividing into 2 or 3 vacuole-like parts (Fig. 10, J, K) as the refringent bodies are lost to view in their granular matrix (Fig. 10, J, L). Eventually the whole protoplasmic mass streams through the germ hypha into a terminal vesicle, where it is fashioned into laterally biciliate zoospores (Fig. 10, M). The empty evacuation tube here commonly measures from 50 to 200 μ in length (Fig. 10, M-Q: t), thus, with respect to this dimension, generally exceeding the corresponding element in *P. oligandrum*. Very often the membrane of the tube is abruptly reflexed at the orifice (Fig. 10, M-P: t), though instances are never lacking where the mouth has no lipped modification (Fig. 10, Q, t). Rather frequently an evacuation tube is found bearing a short branch near its base (Fig. 10, N-P: t), and occasionally 1 or even 2 branches may be found attached farther upward (Fig. 10, Q, t). However, more abundant branching of a germ hypha (Fig. 10, R) usually betokens here, as in allied species, that direct zoosporangial reproduction is no longer possible, and that, instead, the substance of the oospore will be used for mycelial growth. The zoospores produced through oospore germination swim about for some time before they come to rest and round up (Fig. 10, S-Z). In all respects they behave much like the zoospores produced from sporangia of mycelial origin.

MORPHOLOGY AND DEVELOPMENT OF PYTHIUM SALPINGOPHORUM

Although *Pythium salpingophorum* gives rise to vegetative hyphae as wide as 7 μ , it produces such stout hyphae less abundantly than *P. ultimum*, *P. debaryanum*, or *P. irregulare*, with the result that its mycelium, on the whole, looks considerably less coarse than mycelium of any one of the 3 most

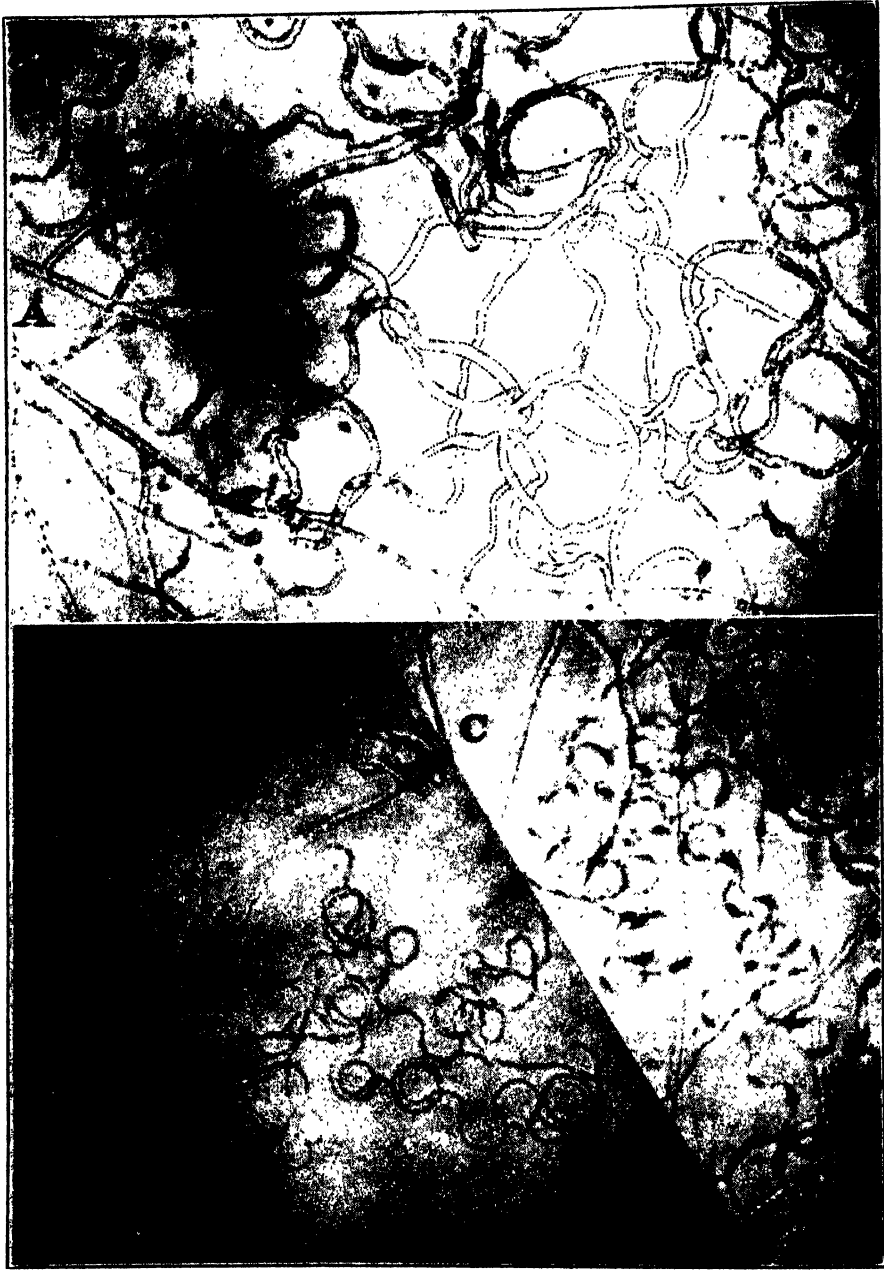


FIG. 11. *Pythium salpingophorum*. A. Mycelium from bottom of a maize meal-agar plate culture, showing thick curving hyphal elements possibly resulting from elongation of functionally frustrated appressoria; the alveolate protoplasmic structure revealed by the hyphal elements being rather usual in aging mycelium of the species. B, C. Tracts of mycelium from upper surface of a maize meal-agar plate culture, displaying elaborate systems of small hyphal coils. Photomicrographs, all approximately $\times 300$. Photomicrograph in A has been retouched.

familiar damping-off species. In maize-meal-agar plate cultures the fungus after several days often displays numerous rather coarse irregular hyphal loops in contact with the glass floor of the Petri dish (Fig. 11, A). Since these loops have about the same width as the appressoria which during somewhat earlier stages of vegetative growth are recognizable as swollen knobs borne terminally on branches of variable lengths (Fig. 12, A, a-d; B, a-d; C, a-g), they might readily be presumed to arise by prolongation of frustrated appressoria. Such a presumption, however, is open to some doubt, inasmuch as the submerged hyphal loops are noticeably coarser than the chains of sickle-shaped structures that can often be observed in meagerly irrigated preparations, and that very obviously come into being through repeated renewal of growth by appressoria unsuccessful in penetrating the glass dish. Further ground for doubt is offered in the usually rather copious production of elaborate hyphal coils (Fig. 11, B, C) on the upper surface of agar cultures, where all development of penetrative organs would be excluded. Although in the main the coils formed on the upper surface are more delicate as well as more intricate than those formed on the glass floor, scattered examples seem to provide transition from one type to the other; thereby suggesting that the submerged coils may in some degree derive from growth tendencies not directly connected with development of appressoria. Whatever their nature may be, the curious hyphal coils, above and below, are often helpful in identifying the species when means are lacking for inducing asexual reproduction.

Like most species of *Pythium* adapted to a terrestrial habitat *Pythium salpingophorum* is capable of producing some zoosporangia under cultural conditions unsuitable for zoospore formation. Thus when grown at 25° C. on maize-meal-agar devoid of liquid water it usually gives rise in the course of 15 to 20 days to a fairly generous scattering of subspherical asexual reproductive bodies (Fig. 13, A, a) resembling with respect to size the familiar conidia of *P. ultimum*. Such globose reproductive bodies, if transferred to a shallow layer of water kept at a temperature near 15° C., soon reveal themselves as zoosporangia by putting forth an evacuation tube (Fig. 13, A, t; B, t) individually. Far more abundant development of sporangia ensues, however, when young well-nourished mycelium is placed under conditions suitable for immediate formation of zoospores. When, for example, slabs are excised from a thin plate culture of maize-meal or Lima bean agar well permeated with actively growing mycelium, and are placed in a shallow layer of water at 15° C., asexual reproductive apparatus (Fig. 13, C-R) will usually be found present in enormous quantity after 10 to 20 hours; the innumerable sporangia then produced, intermingled amid a confusion of active and encysted zoospores, not only blanketing the surface of the slabs but extending out over the narrow fringe of extramatrix mycelium.

Probably the most distinctive morphological character associated with asexual reproduction in *Pythium salpingophorum* is the very pronounced distal widening of the evacuation tube (Fig. 13, C, t; D, t). After the apex

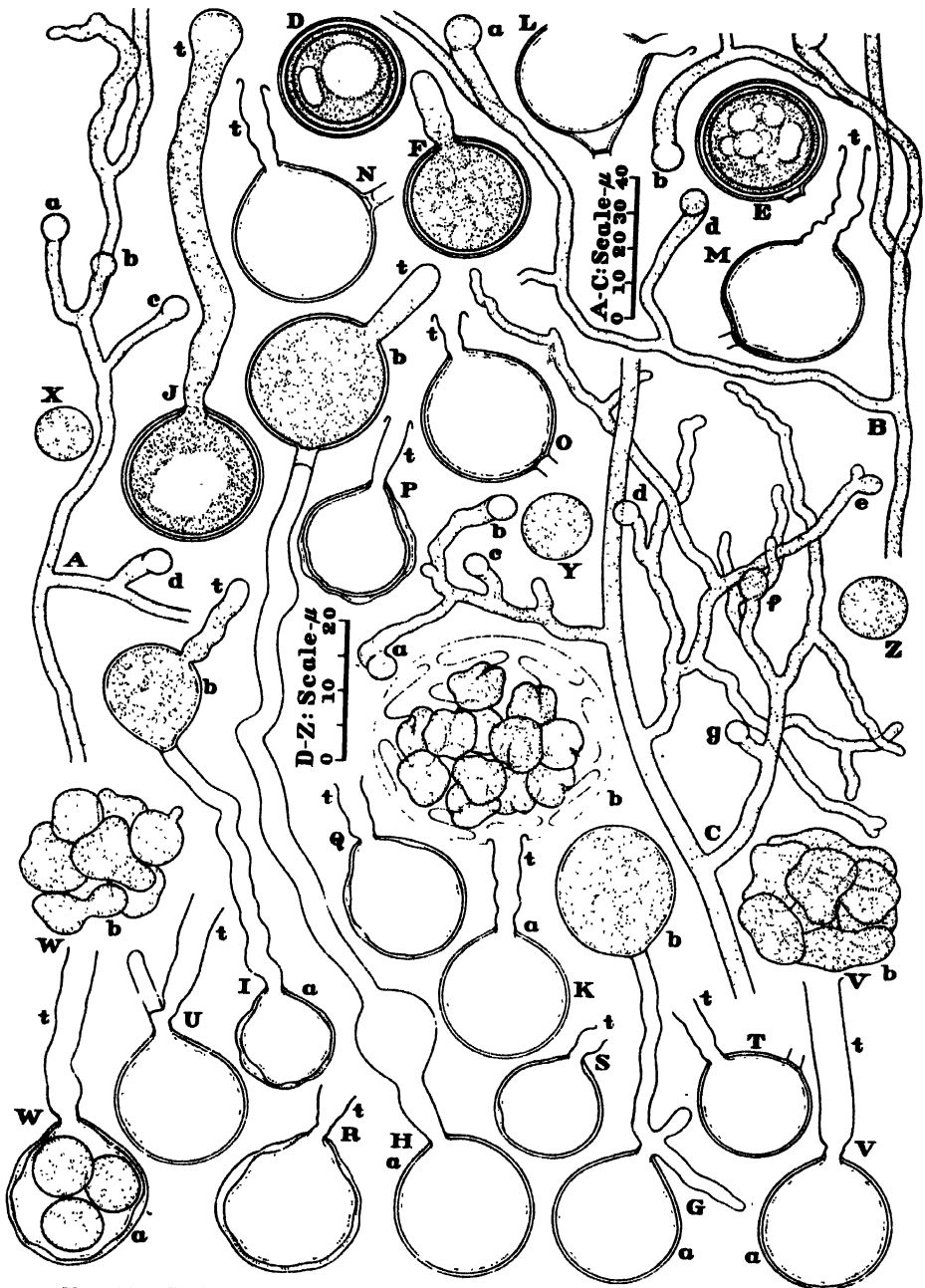


FIG. 12. *Pythium salpingophorum*. A-C. Portions of mycelium at bottom of maize-meal agar plate culture, showing development of appressoria (a-d, a-d, a-g, respectively) in contact with glass floor of Petri dish; $\times 500$. D-Z. Germination of oospores from maize-meal-agar plate cultures 8 months old; drawn with aid of a camera lucida; $\times 1000$. D, E. Oospores shortly after immersion in water, each showing darkening of inner layer of wall preparatory to germination. F. Oospore whose protoplast has assimilated the inner layer of the wall, and is extending a germ hypha, t. G. Oospore, a, that has produced a terminal sporangium, b. H, I. Oospores, a, that have each produced a terminal sporangium, b, from which an evacuation tube, t, has been extended. J. Oospore that has put

of the tube has yielded to permit the sporangial contents to flow out, the flaring terminal portion of the membranous envelope folds backward after the manner of a trumpet with reflexed bell—a feature signalized in the epithet chosen for the species. Where the empty tube is less than 5μ in length, the envelope in its entirety offers a bell-like contour (Fig. 13, E, t; N, t; O, t; P, t). Since the vesicular film is attached, as in other species, to the very rim of the membrane, the rolled anterior portion of the reflexed tube extends perceptibly into the chamber of the vesicle (Fig. 13, G, t; H, t). Generally the vesicle here can be seen more readily than in *P. oligandrum*, being nearly always discernible with good illumination. Even at the time sporangial discharge has just been completed it is usually considerably larger than the mass of loosely enclosed protoplasm. It grows in size as zoospore formation proceeds; so that before the zoospores are ready for liberation its diameter is usually twice the diameter of the empty sporangium (Fig. 13, H, a). In most instances the sporangial envelope, on being evacuated, contracts appreciably in volume, and at the same time takes on the haphazard irregularities of contour frequent in the shrinkage of emptied membranes (Fig. 13, F, a; G-I; K, b; L-N; P); though in some instances the membrane is sturdy enough to maintain its smooth outline after evacuation (Fig. 13, O; J, a, b; K, a). Many of the largest and sturdiest sporangial envelopes (Fig. 13, I; J, a, b; K, a, b) are found in intercalary positions in hyphae lying directly on irrigated portions of substratum. However in irrigated material the most usual position for a sporangium is a subterminal position 3 to 20μ below the tip of a simple or meagerly branched filament; the terminal hyphal part being borne distally on the reproductive body somewhat like an appendage (Fig. 13, D; E; F, b; G; H; M-P). Occasionally where the terminal hyphal part is very short, it is not cut off by a septum; so that the sporangium comes to have a beaked shape, and occupies a terminal position (Fig. 13, F, a). A similar beaked prolongation may at times likewise modify the shape of a sporangium borne more or less laterally (Fig. 13, C). Now and then after a terminal or subterminal sporangium has been evacuated, renewed growth from the basal septum leads to the production of a second sporangium within the emptied envelope (Fig. 13, E); or the supporting hypha may grow out laterally just below the basal septum of the first sporangium (Fig. 13, F, a) to bear a second sporangium (Fig. 13, F, b) on an oblique branch of variable length. In my irrigated preparations of the fungus, proliferous develop-

forth directly an evacuation tube, t, which is about ready to yield at its expanded tip. K. Oospore, a, that has discharged its contents through the evacuation tube, t, into the vesicle, b, where they have been fashioned into 12 zoospores. L-U. Oogonial envelopes, each containing the empty outer layer of the oospore wall, from which the protoplasmic contents have migrated through the evacuation tube, t, to be transformed into zoospores in a vesicle. V. Oogonial envelope, a, surrounding the thin outer layer of the oospore wall which is prolonged into the evacuation tube, t, near the mouth of which 8 immature zoospores have encysted irregularly to form a cluster, b. W. Oogonial envelope, a, surrounding outer layer of oospore wall which encloses 3 well-encysted zoospores; near mouth of evacuation tube, t, is a cluster of 8 irregularly encysted zoospores, b. X-Z. Zoospores that have encysted after period of motility following liberation from vesicle.

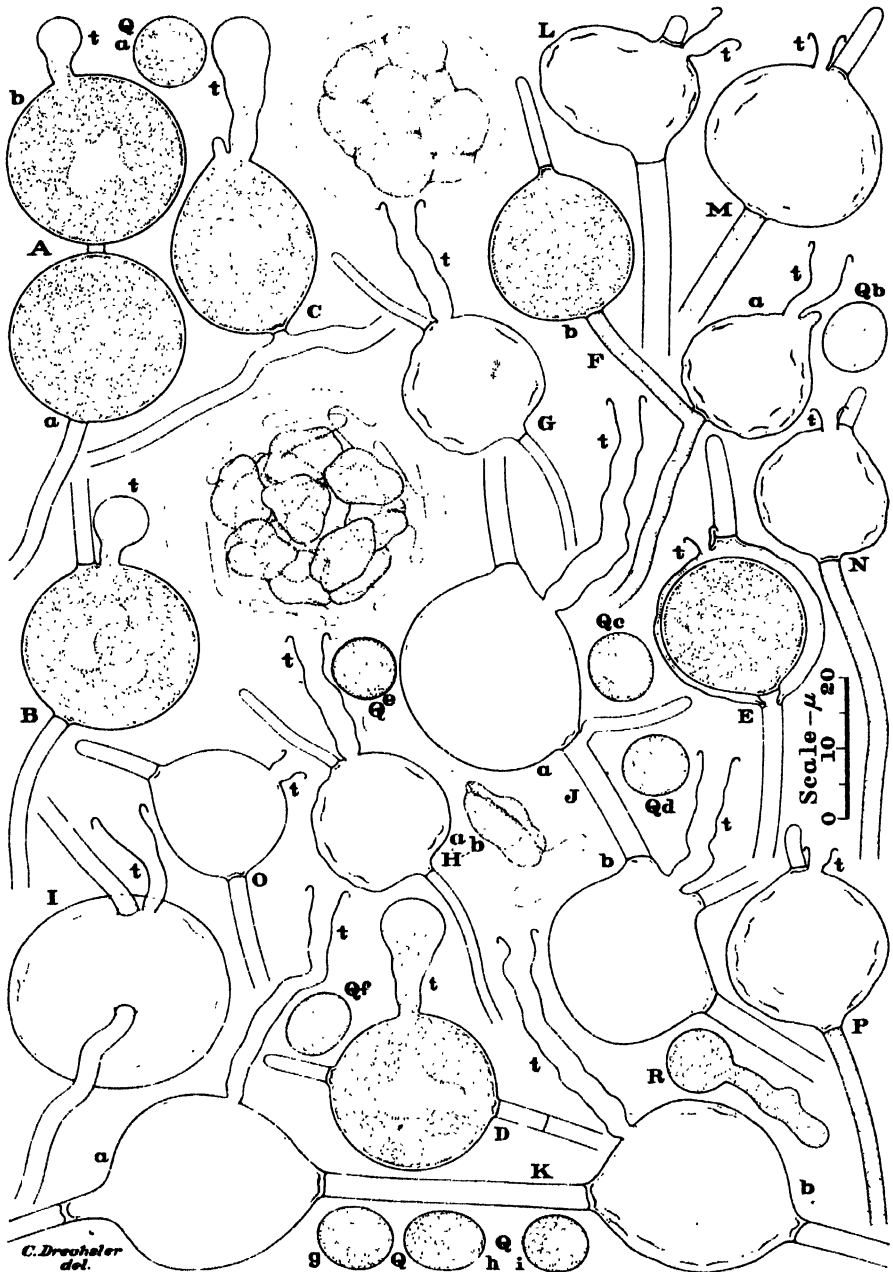


FIG. 13. Asexual reproductive apparatus of *Pythium salpingophorum*, drawn with the aid of a camera lucida from irrigated maize-meal-agar (A, B) and irrigated Lima-bean-agar; $\times 1000$ throughout. A. Two zoosporangia formed close together, one of them, a, in an inert conoidal condition, the other, b, actively extending an evacuation tube. B. Intercalary sporangium actively putting forth an evacuation tube. C. Lateral sporangium with an evacuation tube nearly ready for dehiscence. D. Subterminal sporangium with an evacuation tube ready for dehiscence. E. Subterminal sporangial envelope that after being emptied has become largely occupied by a secondary sporangium. F. Hypha which after producing a terminal sporangium, a, branched distally to bear subterminally a second sporangium, b. G. Subterminal sporangium about 2 minutes after

ment, whether by uniaxial elongation or by subsporangial branching, has always been relatively infrequent. Since, further, such development has been found almost exclusively among the less rangily attached sporangia formed in crowded arrangement on the upper surface of irrigated agar slabs, where observation is far more difficult than in the surrounding extra-matrical fringe, it can hardly be regarded as a feature promising much usefulness in the recognition of the species.

In some irrigated preparations of *Pythium salpingophorum* the actively swimming zoospores (Fig. 13, H, b) have appeared to be slightly longer in proportion to their width than the zoospores of most members of the genus. The difference in shape has, however, not always been clearly observable, and accordingly is not to be urged as a distinguishing feature. On coming to rest the zoospores round up to form subspherical or prolate ellipsoidal cysts (Fig. 13, Q, a-i). Although these cysts usually remain submerged, they have at times been found floating on the surface of the water in countless numbers. They germinate rather readily by putting forth a commonplace germ hypha (Fig. 13, R).

Sexual reproduction takes place freely both in irrigated Lima-bean-agar preparations and in cultures of maize meal agar containing in suspension a substantial quantity of finely divided maize meal. As in *Pythium oligandrum*, parthenogenetic development is frequent. The young oogonia make their appearance here and there on the mycelium as subspherical, prolate ellipsoidal, or oblate ellipsoidal enlargements. Often when two or three are formed adjacent to one another, and no antheridium is present, they look at first much like conidia; their character as oogonia, however, soon becomes evident when, after they have attained definitive size, their protoplasmic contents assume a coarsely lumpy texture (Fig. 14, A, a-c). A thick, spherical oospore wall is then laid down in intimate contact with the inflated portion of oogonial envelope (Fig. 14, B, a, b); the wall as a rule being physically separated from the envelope only where the oogonium is extended at either end. A number of reserve globules thereupon become visible in the midst of the protoplasmic lumps (Fig. 14, B, a, b). Later these are united into a single reserve globule; and the surrounding lumps are resolved into minute granules to be distributed as components of the parietal layer in which a single refringent body of orbicular or oblate ellipsoidal shape emerges clearly into view. The resulting parthenospore when fully mature (Fig. 14, C; D, a-c; E, a-c; F, a-c; G, a-c; H, a-d) thus reveals the unitary internal organization characteristic of oospores in most members of the genus.

Although parthenogenesis often predominates over conjugative development in *Pythium salpingophorum*, it is common for 1 in 3 or 4 oogonia to be

discharge of contents into vesicle. II. Same sporangium about 15 minutes after discharge, showing 14 zoospores within vesicle nearly ready for liberation; b, zoospore after liberation. I; J, a, b; K, a, b. Empty envelopes of intercalary sporangia, each bearing a rather long, empty evacuation tube. L-P. Empty envelopes of subterminal sporangia, each bearing a rather short, empty evacuation tube. Q. Encysted zoospores, a-i, showing variation in size and shape. R. Germinating zoospore. (t, evacuation tube.)

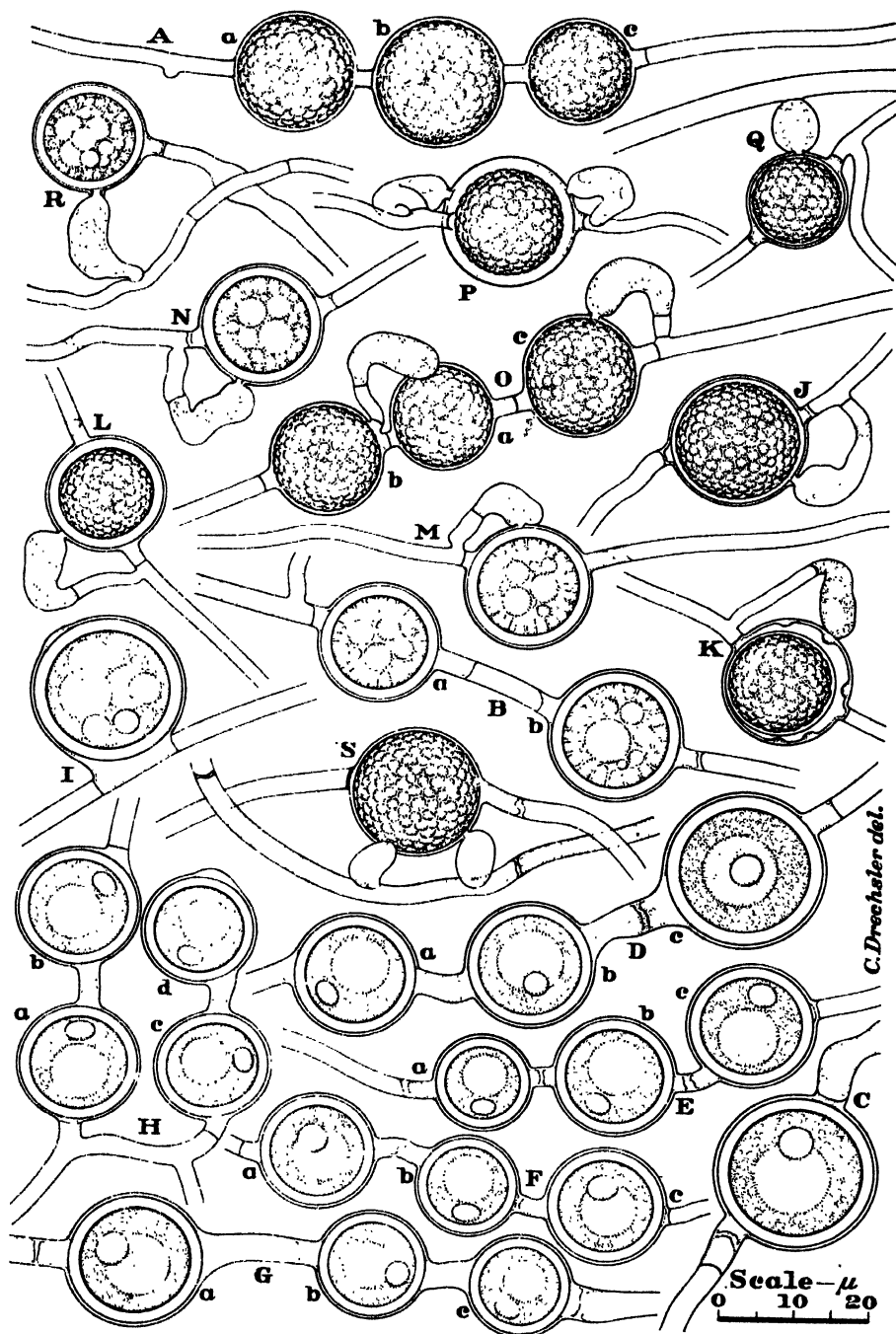


FIG. 14. Sexual reproductive apparatus of *Pythium salpingophorum* drawn from irrigated Lima-lean-agar (A, B, K-S) and from maize meal-agar plate cultures (C-J) with the aid of a camera lucida; $\times 1000$ throughout. A. Hypha bearing 3 young but full-grown oogonia, a and b, each containing an immature parthenospore with thick wall. B. Hypha bearing intercalary oogonium of large size, with a mature parthenospore. C. Intercalary oogonium of large size, with a mature parthenospore. D-G. Portions of hyphae, each bearing 3 mature parthenospores, a-c. H. Portion of mycelium bearing 4 mature parthenospores, a-d. I. Laterally intercalary oogonium with large parthenospore still

supplied with a male complement. Most frequently this complement consists of a single clavate crook-necked antheridium (Fig. 14, J-N; O, a, c) borne on a short branch arising from the oogonial hypha either in immediate proximity to the oogonium (Fig. 14, K-N; O, a, c) or occasionally at a distance of several microns from the oogonial boundary (Fig. 14, J). Now and then an androgynous branch antheridium is supplied from both the proximal and the distal side of the oogonium (Fig. 14, P). Occasionally, again, an antheridium (Fig. 14, Q, R) or 2 antheridia (Fig. 14, S) are contributed by a hypha having no close mycelial connection with the oogonium; male cells of such origin being more often sessile than those of androgynous origin, and usually lacking crook-necked curvature. As a rule the oogonial envelope becomes noticeably lipped about the short fertilization tube usually extended from the apex of the antheridium. In most instances a substantial portion, if not all, of the antheridial contents are delivered through the tube. Failure of fertilization may be inferred in other instances (Fig. 14, K, O, a), where no tube is intruded or where the antheridium retains its contents undiminished. Here and there an oogonium (Fig. 14, O, b) may be found to which is directly attached an antheridial branch supplying an adjacent older oogonium (Fig. 14, O, a); an appearance being thereby presented as if the antheridial branch were of oogonial origin. There is much reason to presume, however, that in all such cases the antheridial branch grew out from a portion of undifferentiated hyphal filament, and that it came into its anomalous positional relationship subsequently when the hyphal portion was distended to form the younger of the 2 contiguous oogonia. A similar relationship of parts is frequent in my *P. paroecandrum* (22, p. 208) and has been observed likewise in some cultures of the familiar *P. ultimum* that produced sexual apparatus very abundantly.

In irrigated preparations of *Pythium salpingophorum* scattered oogonia may be found enclosing oospores so much smaller (Fig. 14, K) that separation between oogonial envelope and oospore wall is no less distinct than in *P. debaryanum* or *P. ultimum*. However, in agar cultures such separation is usually evident only where the spherical contour of the oogonium merges with the cylindrical contour of the supporting filament; though here and there, especially in terminal (Fig. 14, H, d) or laterally intercalary (Fig. 14, I) oogonia, separation may be observable, besides, in blister-like irregularities of the oogonial envelope. For the most part, oospores of the species have little the aspect of endogenous reproductive bodies. On aging,

slightly immature with respect to distribution of reserve material among several globules. J. Oogonium fertilized by a branch antheridium arising nearby from same hypha; oospore in early stage of development. K. Oogonium apparently not fertilized though supplied with a branch antheridium arising nearby from the same hypha; the parthenospore here being unusual in lying loose within the oogonial chamber. L-N. Solitary oogonia, each supplied with an antheridium arising nearby from same hypha; the oospores here showing successively later stages in maturation. O. Three adjacent oogonia, one of them, a, supplied with an antheridium on a branch from its younger neighbor, b, while the third, c, is fertilized by an antheridium originating nearby from the parent hypha. P. Oogonium supplied with 2 antheridia, both borne sessile on the oogonial filament. Q, R. Oogonia, each supplied with an antheridium borne sessile on a neighboring hypha. S. Oogonium supplied with 2 antheridia sessile on a neighboring hypha.

after all antheridia have vanished from sight, they could readily be mistaken for chlamydospores, were it not for their internal organization. They show generally a fairly high degree of uniformity with respect to size. The metric data, given in the diagnosis, relative to oogonium and oospore were based on 200 measurements of specimens chosen at random in 14-day-old maize-meal-agar plate cultures containing very abundant sexual apparatus with virtually no degeneration. The 200 oogonia gave values for diameter, expressed in the nearest integral number of microns, distributable as follows: 11 μ , 1; 12 μ , 1; 13 μ , 10; 14 μ , 27; 15 μ , 42; 16 μ , 54; 17 μ , 41; 18 μ , 14; 19 μ , 6; 20 μ , 2; 21 μ , 1; 22 μ , 1; and the 200 oospores or parthenospores, all of correct internal structure, that were contained in them, gave values for diameter, expressed in the nearest integral number of microns, distributable thus: 10 μ , 1; 11 μ , 1; 12 μ , 13; 13 μ , 29; 14 μ , 40; 15 μ , 62; 16 μ , 33; 17 μ , 11; 18 μ , 6; 19 μ , 4.

Oospores from maize-meal-agar plate culture 250 days old were found to germinate freely when placed in a shallow layer of water kept at temperatures near 16° C. During the period of after-ripening the oospore wall becomes more distinctly differentiated into an outer colorless layer and a somewhat thicker yellowish inner layer. On immersion in water the differentiation is further accentuated through radial markings of the inner layer (Fig. 12, D). In some cases the single reserve globule now divides into several globules, and the refringent body also may undergo division (Fig. 12, E). Soon the inner layer of the oospore wall merges indistinguishably with the protoplast, which puts forth a protrusion that after pushing through both the outer layer of the oospore wall and the oogonial envelope continues growth externally as a germ tube (Fig. 12, F); the reserve globules and the 1 or 2 refringent bodies meanwhile being lost to view. Sometimes after the germ hypha has attained a length of 25 to 100 μ , all the protoplasmic contents of the oospore (Fig. 12, G-I: a) are utilized for the production of a terminal sporangium (Fig. 12, G-I: b), which may later put forth an evacuation tube (Fig. 12, H, t; I, t) much like a sporangium of mycelial origin. However, under favorable conditions the oospore (Fig. 12, J; K, a) functions directly as a sporangium; the germ tube extended by it (Fig. 12, J, t) forming on its expanded tip a cap of dehiscence which on yielding permits migration of the oospore contents into a terminal vesicle where they are fashioned into laterally biciliate zoospores, mostly 6 to 15 in number (Fig. 12, K, b). The empty evacuation tubes here are often rather strongly reflexed at the end (Fig. 12, L-P: t), though frequently, too, they are merely widened at the mouth without being folded backward (Fig. 12, Q-V: t), and occasionally they show no distal widening (Fig. 12, W, t). Owing very probably to lack of sufficient water the vesicle sometimes disintegrates prematurely, with the result that the young zoospores, not yet provided with flagella, encyst in irregular shapes and thus remain clustered near the mouth of the evacuation tube (Fig. 12, V, b; W, b). Where a portion of protoplasm fails to migrate from the chamber of the

oospore, it is nevertheless successfully fashioned into zoospores, which after a period of movement within their small enclosure round up to form spherical cysts (Fig. 12, W, a) indistinguishable from the cysts formed from normally liberated zoospores (Fig. 12, X, Y, Z). After being emptied of contents the persistent thin outer layer of the oospore wall sometimes remains appressed to the oogonial envelope (Fig. 12, G, H, K, N, O, T, U, V), but at other times shrinks away to become more clearly visible as a discrete membrane (Fig. 12, I, M, P, Q, R, S, W).

Like many congeneric forms *Pythium salpingophorum* is subject to severe attack when it is grown on maizemal agar in dual culture with *P. oligandrum*. Its mycelium is abruptly halted in its advance when it encounters growing mycelium of the echinulate species; the extension of the echinulate species, however, continuing without interruption. Everywhere in the zone of encounter young hyphae of *P. salpingophorum* (Fig. 15, A, a; B, a) become elaborately enveloped by branches of *P. oligandrum* (Fig. 15, A, b; B, b). Numerous threads of the smooth species (Fig. 15, C, a) are extensively invaded by assimilative filaments of the spiny one (Fig. 15, C, b), although plugs and irregular septa laid down in the former have some effect in restricting the field of each invasion. After the contents of the host thread (Fig. 15, D, a) have been appropriated, the assimilative filaments (Fig. 15, D, b) extend branches through the hyphal membrane to attack other threads. In dual culture with *P. acanthicum*, the mycelial advance of *P. salpingophorum* is likewise stopped abruptly in the zone of encounter; and its young hyphae (Fig. 15, E, a) here similarly become enveloped by branches of the opponent mycelium (Fig. 15, E, c). While large hyphae of the smooth form (Fig. 15, F, a) sometimes successfully resist invasion by the enveloping branches (Fig. 15, F, c), their protoplasmic contents nevertheless suffer thoroughgoing degeneration. Hyphae of *P. salpingophorum* having less indurated walls (Fig. 15, G, a; H, a) are readily invaded by *P. acanthicum* (Fig. 15, G, c; H, c) and when their contents have been appropriated (Fig. 15, I, a) the internal haustorial filaments (Fig. 15, I, c) erupt through the enveloping membrane to begin another attack. Similar injury is incurred by *P. salpingophorum* when it is grown in dual culture with *P. periplocum*; the advance of its mycelium being sharply arrested on meeting the opponent mycelium. Here, also, in the zone of encounter numerous hyphae of *P. salpingophorum* (Fig. 15, J-O: a) are penetrated by branches of the echinulate species (Fig. 15, J-O: d) and invaded lengthwise by assimilative filaments. Later these filaments often push branches (Fig. 15, P, d) through the membrane of the host (Fig. 15, P, a) to extend the destructive relationship to other hyphae of the smooth species.

In diseased pea roots from which it was originally described and from which it has since been isolated by Horsfall and Kertesz (29), *Pythium salpingophorum* is frequently found associated with the saprolegniaceous parasite *Aphanomyces euteiches* Drechs. As it was isolated from spinach

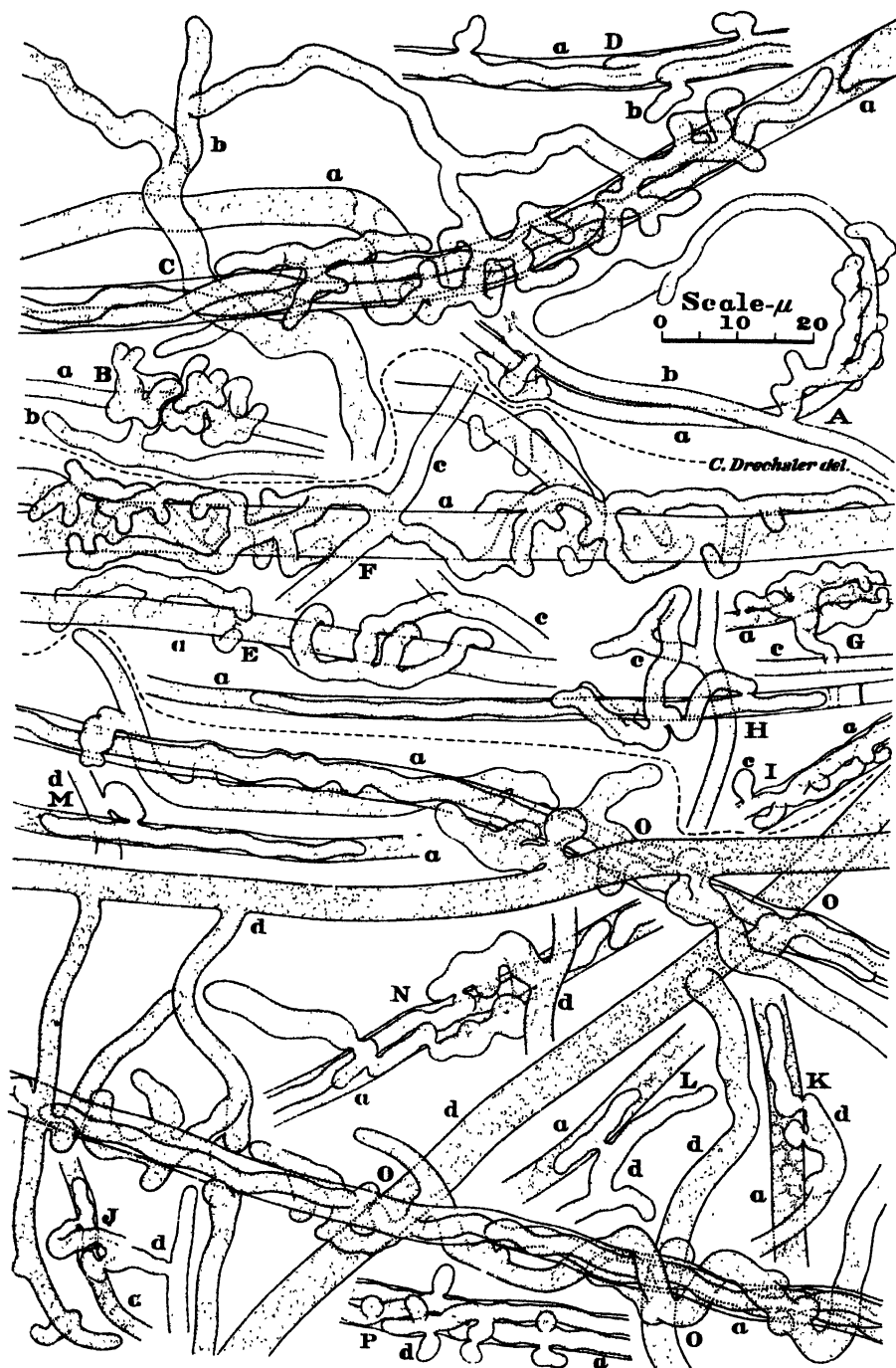


FIG. 15. Drawn from maize meal-agar plate cultures with the aid of a camera lucida; $\times 1000$ throughout. A-D. Hyphae of *Pythium salpingophorum*, a, attacked by *P. oligandrum*, b. E-I. Hyphae of *P. salpingophorum*, a, attacked by *P. acanthicum*, c. J-P. Hyphae of *P. salpingophorum*, a, attacked by *P. periplocum*, d.

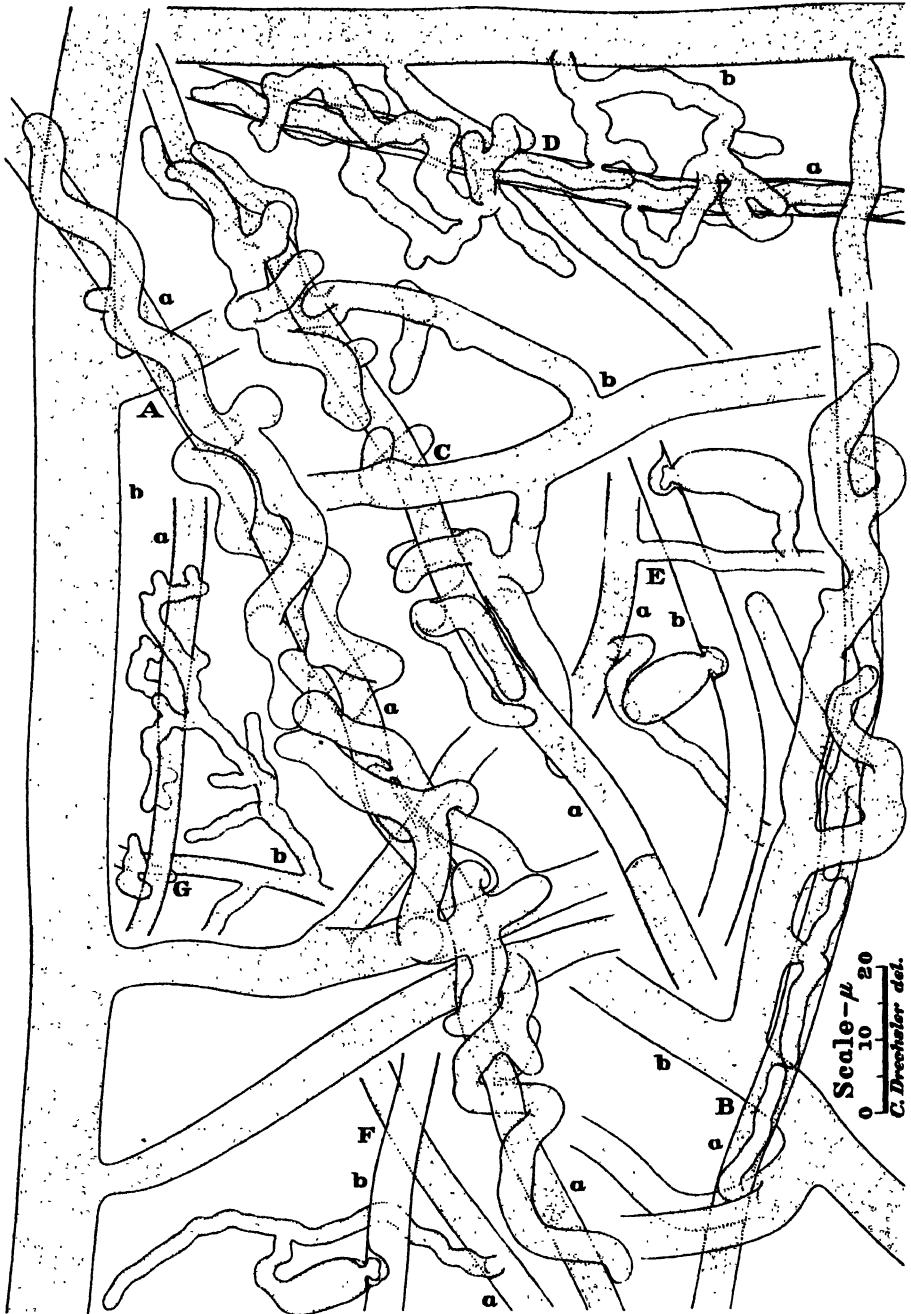


FIG. 16. Drawn from maize meal-agar plate cultures with the aid of a camera lucida; $\times 1000$ throughout. A, B. Hyphae of *Pythium salpingophorum*, a, attacked by *Aphanomyces cladogamus* (spinach strain), b. C. Hypha of *Pythium vexans*, a, attacked by *Plectospora myriandra*, b. D. Hypha of *Pythium vexans*, a, attacked by *Pythium periplocum*, b. E, F. *Pythium vexans*, a, attacking *Pythium periplocum*, b, by means of appressoria. G. *Pythium vexans*, a, attacked by *Pythium acanthicum*, b.

(*Spinacia oleracea* L.) roots collected near Norfolk, Va., late in November, 1932, and from tomato rootlets collected near Beltsville, Md., in September, 1942, *P. salpingophorum* is known to occur on at least 2 phanerogamic crop plants that have been recorded as hosts of another saprolegniaceous root-rotting species, *A. cladogamus* Drechsl. (15, 16, 19). When the fungus is grown in opposition to *A. cladogamus* on maize meal agar, its mycelial advance is abruptly halted where it encounters the mycelium of the water mold. Many of its hyphae (Fig. 16, A, a; B, a) in the zone of encounter are soon copiously involved by branches extended from filaments of *A. cladogamus* (Fig. 16, A, b; B, b), their protoplasmic contents promptly showing degenerative changes by taking on a darkish opaque, lumpy appearance. Here and there the *Aphanomyces* branches narrowly penetrate into the *Pythium* filaments and intrude assimilative hyphae to appropriate the degenerating materials (Fig. 16, A, B).

THE MORPHOLOGY AND IDENTITY OF *PYTHIUM VEXANS*

In 1876 de Bary (2) briefly described under the binomial *Pythium vexans* a fungus which he first found in the month of July in dead exhausted cells of several potato tubers that had sprouted despite infection with *Phytophthora infestans*; the specific epithet being chosen because for two long years the fungus had given trouble with respect, more especially, to its separation from the late blight parasite. Five years later he (4) expanded his earlier account by adding a few more illustrations and by supplying further descriptive details, most of which provided contrast with *Pythium debaryanum*. Spherical monosporous oogonia, in most instances mature, were set forth as having been found attached laterally to delicate branched hyphae that could be traced into intercellular spaces of the spent tuber; the globose organ sometimes being borne on a very short stalk, sometimes being sessile on the parent filament, and sometimes, again, being broadly inserted upon the parent filament as a tangentially intercalary body. The single antheridium usually comprising the male complement—2 antheridia were present only rarely—was described as being mostly of curved clavate shape and as arising from the oogonial hypha in immediate proximity to the oogonium. Considerably smaller size of oogonium and oospore was represented as a feature separating the species from *P. debaryanum*; the diameter of the former structure having been determined from permanent mounts as 15 to 18 μ , that of the latter structure as 12 to 15 μ . The fungus was held distinguished from *P. debaryanum* also by the greater delicateness of its oogonial envelope, by greater size of its oospore in relation to the oogonium, by germination of its oospores after only a brief resting period, and by its inability in repeated trials to infect tissues of the potato plant. It gave further manifestations of saprophytism by growing luxuriantly on dead flies and on dead mites; within the mites it produced oospores, but within the flies it gave rise only to branching mycelial hyphae and to spherical conidia like those of *P. debaryanum*. In one of de Bary's figures (4: Taf. V, fig. 3)

wherein a portion of mycelium is shown, the main axial hypha would seem, from the magnification indicated in the legend, to vary in width from 3.5 to 4 μ , while a secondary branch would seem to have a width of about 1.5 μ . On this narrow branch is shown attached a unit of sexual apparatus that reveals in profile view broad application of an antheridium to a flattened portion of the oogonial wall; the portion of antheridial envelope not adnate to the oogonium presenting a semicircular contour. In a figure of another unit of sexual apparatus (4: Taf. V, fig. 4) the antheridium is shown advantageously in dorsal view as being extensively applied flatwise to the upper aspect of the oogonium; its attachment to a very short stalk arising ostensibly from the oogonial hypha in immediate proximity to the oogonium, and its divaricately bilobate shape, seeming especially worthy of attention.

Butler's account of *Pythium vexans* (10, p. 91-94) consists mainly of a first-hand description of a fungus which he found not uncommon in garden soil in Great Britain and France, and which he considered undoubtedly the same as de Bary's largely because of distinctive peculiarities he recognized in the frequently broad insertion of the oogonium and in the clavate or rounded shape of the relatively large antheridial cell. In *Abutilon*-root cultures the fungus gave rise extramatrically to oospores 20 to 22 μ in diameter within oogonia measuring 22 to 25 μ in diameter; its main measurements, therefore, not only considerably exceeding those originally ascribed to *P. vexans*, but also exceeding, even if only rather slightly, those ascribed to *P. debaryanum* (or to the synonymous *P. Equiseti* Sadebeck) both by de Bary (21 to 24 μ for oogonial diameter and 15 to 18 μ for diameter of oospore) and by Butler (20 to 25 μ for oogonial diameter and 14 to 18 μ for diameter of oospore). The individual oogonium was reported to be supplied with one antheridium, rarely with two; the antheridia usually arising from the oogonial stalk and sometimes being hypogynal. De Bary's account of *P. vexans* makes no mention of hypogynal antheridia, nor of an arrangement of sex organs which Butler found to be fairly common—an arrangement initiated through prolongation of the oogonial branch from below the female cell as a somewhat coiled, plurally diverticulate stalk which then cuts off terminally an antheridial cell that bends around to reach the apex of the oogonium and intrudes there a fertilization tube. Butler found the antheridial cell always closely applied to the oogonial wall so as to fuse with this wall over a large part of its circumference; the two conjugating organs together appearing commonly as a pear-shaped bilocular structure. In his figures of such structures (10: Plate V, fig. 8, a, b; 9) the male component is shown with a dome-like profile rather similar, it must be admitted, to the semicircular antheridial profile drawn by de Bary. While in some of his cultures Butler obtained zoosporangia in addition to conidia—both measuring 17 to 24 μ in diameter—other cultures yielded conidia but no zoosporangia; so that the fuller scope of asexual reproductive development in his fungus was readily reconcilable with de Bary's findings. Some departure from the conidial morphology implied in de Bary's account might not wholly

without reason be read into Butler's statements intimating that the conidia in his material were much more frequently of irregularly pyriform, ovoid, or subangular shape than of more symmetrical subspherical shape, and that their protoplasmic contents were of denser appearance than the contents of conidia in *P. debaryanum*. A vegetative character distinguishing his fungus from any other species known to him was recognized by Butler in the frequent prolongation of secondary and tertiary branches far beyond the primary hyphae, and their attenuation at the ends into very fine filaments. Lateral branches, according to his statement, were given off in a very irregular manner; and the mycelium, in general, was slender, finer than that of *P. debaryanum*.

In 1924 Braun (8) described as a new species, under the binominal *Pythium complectens*, a fungus he had isolated from blackened geranium (*Pelargonium sp.*) stems found in greenhouses at Washington, D. C., which in inoculation experiments caused decay in the stems of geranium cuttings and in *Coleus* cuttings, though it failed to attack either cucumber or watercress seedlings, and in radish (*Raphanus sativus* L.) seedlings caused only superficial black streaks on the stems. Without determining whether his fungus would attack the living potato plant—the only phanerogam de Bary tried out in the experimentation relevant here—Braun distinguished *P. complectens* from *P. vexans* partly on the score of its pathogenicity. He distinguished his fungus in part, again, on the ground that its hyphae, measuring 1.7 to 4.85 μ in width, were cylindrical with rounded tips and did not taper to fine points; although it would seem by no means certain that the very fine filaments of Butler's fungus need necessarily have been sharply pointed at the tip, or that the plant bearing them was actually referable to *P. vexans*. Owing to the uncertain identity of Butler's plant, the contrast that its rare, irregularly shaped sporangia offer to the subspherical or oval sporangia produced abundantly on various agar media by the geranium fungus appears of dubious relevance in separating this fungus from *P. vexans*. Braun assuredly erred in representing de Bary as having observed no sporangia or conidia in *P. vexans*; for, as has been mentioned, de Bary reported development of conidia when his species was grown on flies. Indeed, since the German mycologist likened his conidia to those of *P. debaryanum* they must have been rather similar to the sporangia Braun ascribed to *P. complectens*, which in shape and size—their diameter being stated to vary from 16.4 to 27.3 μ —show moderate resemblance to the conidia of *P. debaryanum* and of *P. ultimum*. According to Braun, the oogonia of the geranium parasite are borne each on a slender stalk, and are not inserted by a broad base into the mycelial tube after the manner of oogonia in *P. vexans*. The antheridium of his fungus, an organ described as "single, one-celled, arising from adjacent hypha or below oogonial stalk, persistent, varying from a trumpet shape flaring out at region of attachment, to a broad irregularly lobed mass clasping a large part of the oogonium and fused with it," Braun recognized as resembling in shape the broadly applied anther-

idium by which, in his view, de Bary characterized *P. vexans*. From his qualifying comment to the effect that a figure of de Bary's (4: Tab. V, fig. 3) shows, nevertheless, one clavate antheridium, it would seem that Braun regarded clavate antheridial shape in *P. vexans* as a feature rather exceptional in the morphology originally ascribed to the species by its author. In reality, however, de Bary set forth the antheridium of *P. vexans* as being usually of curved clavate shape and only seldom of other form. Somewhat curiously, therefore, de Bary's statement of antheridial morphology might have afforded a stronger argument in favor of separating the geranium pathogen from *P. vexans* than was derived from it by Braun.

After the publication of the paper presenting *Pythium complectens* as a new species, Braun kindly gave me a culture of his fungus. By comparison of material grown under like conditions, the fungus was readily seen to be identical with more than a dozen cultures sorted out, mainly because of resemblances in mycelial luster and antheridial morphology, from a numerous collection obtained in 1924 from softened pea roots and blackened sweet-potato rootlets—from the same collection in which, as has been noted, *P. oligandrum* was found so abundantly represented. The species has subsequently been recognized in cultures isolated from affected roots of pansies, tomatoes, peppers (*Capsicum annuum* L.), beans, giant ragweed plants, and pale touch-me-not plants collected in Arlington, Va., in Washington, D. C., and near Beltsville, Md. It has been recognized likewise in several cultures isolated from sugar-beet roots collected near East Lansing, Mich., and near Saginaw, Mich., in June, 1927, as well as in a few cultures among a much larger number isolated from celery (*Apium graveolens* L.) seedlings then collected near Kalamazoo, Mich. Later it was encountered also in a few cultures derived from discolored spinach rootlets gathered in fields near Norfolk, Va., in November, 1932.

In maize-meal-agar plate cultures the fungus produces a mycelium of markedly lustrous radiating appearance attributable here as in allied forms to a rather pronounced degree of parallelism in arrangement of the submerged and prostrate axial hyphae. This type of appearance, to which Braun aptly refers as a "combed silk effect," and which he illustrates rather satisfactorily (8: Plate 1, b), may justly be considered well worth mentioning in descriptive accounts, even though its presence in similar cultures of various congeneric species, including, for example, *Pythium complens* Fischer and *P. acanthicum*, somewhat abates its distinctiveness as a diagnostic character. Aerial mycelium is frequently absent on maize-meal agar in Petri-plate cultures, but usually is produced in moderate quantity on the same substratum in tube cultures. Somewhat more abundant aerial development ensues when a richer medium like Lima-bean agar is employed. In this medium the submerged mycelium reveals at times some cumulous variegation in density of hyphal elements.

After its vegetative growth in maize-meal-agar plate cultures has been concluded, the fungus begins to produce, as a rule, both asexual and sexual

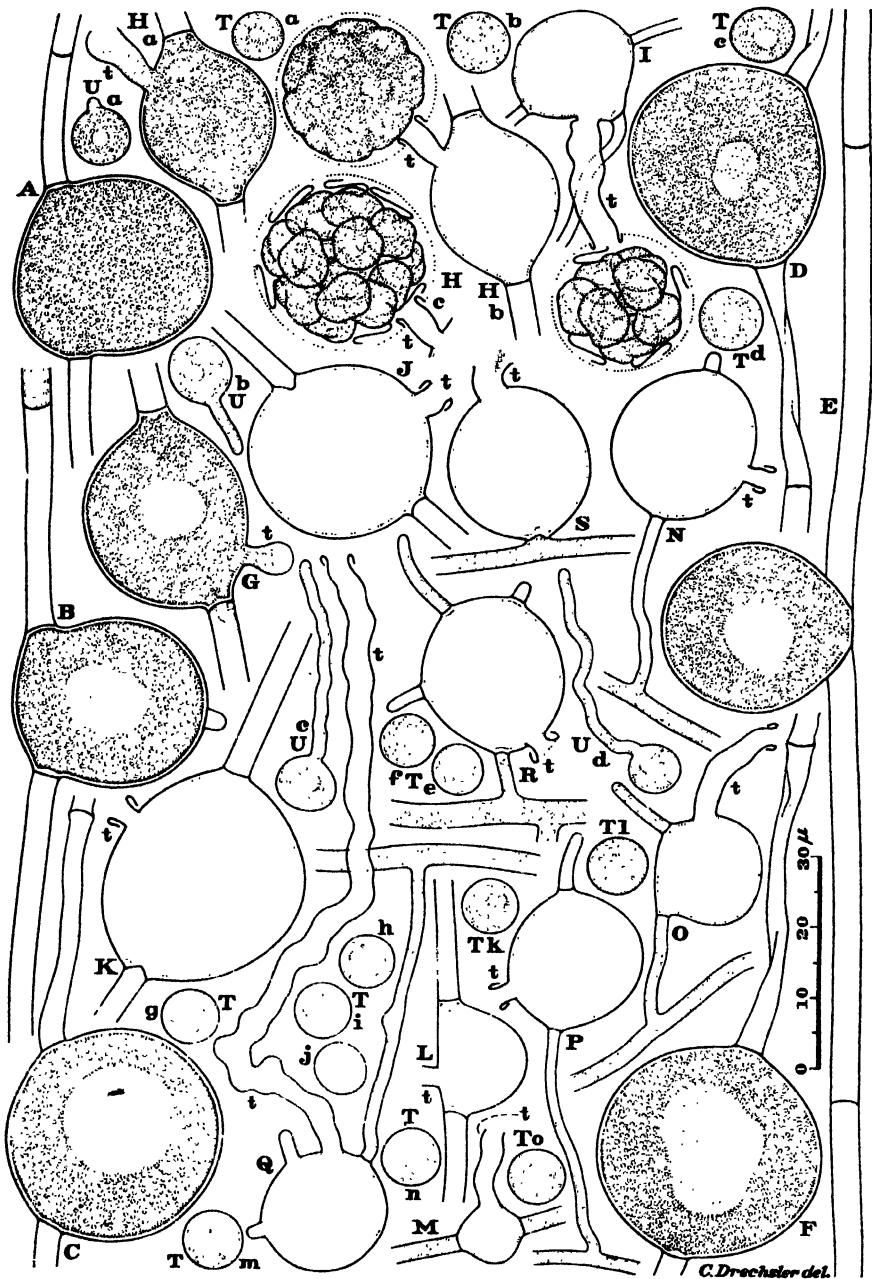


FIG. 17. Asexual reproductive apparatus of *Pythium vexans* drawn with the aid of a camera lucida from a maize-meal-agar plate culture (A-F), from irrigated maize-meal agar (G-K), and from irrigated Lima-bean agar (L-U); $\times 1000$ throughout. A-F. Intercalary sporangia as found in rather soft maize-meal-agar plate cultures 51 days after inoculation. G. Sporangium that was formed in a maize-meal-agar culture, and that extended an evacuation tube promptly on addition of water. H. Sporangium, a, that was formed in a maize-meal-agar plate culture and that promptly extended an evacuation tube on addition of water; b, same sporangium after discharge of granular contents into a vesicle; c, empty, distally reflexed evacuation tube of same sporangium, together with the

reproductive apparatus. Since in such cultures zoospore formation cannot take place owing to lack of free liquid water, asexual reproduction is restricted to development of subspherical or ellipsoidal bodies mostly varying in diameter between 15 and 30 μ . In cultures 15 to 25 days old a substantial proportion of these bodies often measure from 25 to 30 μ in diameter. For the most part specimens of such large size are found in laterally intercalary (Fig. 17, A-E) or mesially intercalary (Fig. 17, F) positions in the stouter main mycelial filaments; thus offering, with respect to size and hyphal relationships, the similarity to conidia of *Pythium debaryanum* and *P. ultimum* that is to be inferred from de Bary's account of *P. vexans*. When the globose bodies are sparingly irrigated with distilled water they usually germinate rather promptly. Sometimes germination takes place almost exclusively by emission of germ tubes that grow directly into branched mycelia; whereas at other times by far the greater number of globose bodies give rise to zoospores. The frequent predominance of the one or the other of the alternative modes of development affords some measure of reconciliation between the findings of de Bary and of Braun relative to asexual reproduction.

Butler's statement that in *Pythium vexans* zoospores are given only when sporangia are sown immediately in fresh water would seem to imply that de Bary's failure to obtain zoospore development could well have been due to the age of the conidia in his material—an implication not necessarily devoid of merit because of the questionable identity of Butler's fungus. Braun reported that in *P. complectens* the asexual reproductive bodies gave rise to zoospores for 10 days after their formation; the proportion of bodies that produced zoospores when placed under circumstances favorable for germination thereafter diminishing with increasing age. My cultures of the species have shown at times even more enduring capacity for zoosporangial development. Thus, although the reproductive bodies shown in figure 17, A-G, which were drawn from a Petri-plate culture prepared with rather soft, slightly moist agar 51 days after planting, mostly showed clear evidence of aging in the presence of a central vacuole (Fig. 17, B-G), all of them and the generality of their very numerous fellows germinated as sporangia on addition of a small quantity of distilled water. In this material, moreover, germination began very promptly. Many of the globose bodies were observed individually putting forth an evacuation tube (Fig. 17, G, t; H a, t) within 10 minutes after the water had been added; exten-

vesicle shortly before the motile zoospores escaped. I. A smaller intercalary sporangium which was formed in a maize-meat-agar plate culture, and which on addition of water promptly extended an evacuation tube and discharged its contents into a vesicle for transformation into 8 zoospores. J, K. Empty envelopes left after irrigation of large intercalary sporangia from a maize-meat-agar plate culture; each envelope bearing a short reflexed evacuation tube. L-S. Empty envelopes of sporangia that were formed and discharged in an irrigated Lima-bean-agar preparation; illustrating intercalary (L, M), subterminal (N, O, P, R), terminal (Q), and lateral (S) positional relationships to supporting hyphae; and showing plain-rimmed (L, M, S) and reflexed (N-R) conditions in empty evacuation tubes, as well as pronounced variation in length of these tubes. T. Encysted zoospores, a-o, showing variations in size and shape. U. Zoospores, a-d, each germinating by production of a germ tube. (t, evacuation tube.)

sion of the tube to its definitive length in the course of the ensuing 15 minutes being followed by abrupt yielding of its hyaline expanded tip and by migration of the sporangial contents into a terminal vesicle (Fig. 17, H b, t) for conversion into motile zoospores (Fig. 17, H c; I). Within 45 minutes after addition of the distilled water zoospores were observed swimming about in easily noticeable numbers; in 3 hours they were swarming abundantly throughout the irrigated preparation. Of the numerous sporangia a large proportion were now represented only by empty envelopes (Fig. 17, J, K), each provided with an empty evacuation tube (Fig. 17, J, t; K, t).

Zoospore development can readily be induced in the species, as in most congeneric forms, by excising from maize-meal-agar or Lima-bean-agar plate cultures thin slabs well permeated with vigorous mycelium and transferring them to a thin layer of water. In such preparations sporangia and motile zoospores make their appearance in moderate numbers after about 24 hours, and with occasional renewal of water will ordinarily continue to be formed in some quantity for several days. Since the conditions necessary for zoospore development are here constantly present, the sporangia fail to attain generally as large a size as in agar cultures devoid of free water; their transverse diameter varying usually from 8 to 23 μ (Fig. 17, L-S). While many are found in intercalary positions (Fig. 17, L, M), others are produced subterminally on relatively slender branches, so that a terminal portion of the branch is borne somewhat like a distal appendage (Fig. 17, N, O, P). Sometimes the distal portion of the supporting branch does not become delimited by a septum, and then will appear as a diverticulum of a terminal sporangium (Fig. 17, Q). In other instances not only the distal portion of the supporting branch but also 1 or 2 short lateral secondary branches are each cut off by a septum, and thus likewise come to be borne on the subterminal sporangium as appendages (Fig. 17, R). Occasionally a sporangium is borne laterally on an axial filament (Fig. 17, S).

In sparingly irrigated material, where the sporangia are not deeply submerged and yet are adequately bathed in a thin layer of water so that the positional relationships to water and air are nearly everywhere favorable for zoospore development, the evacuation tube usually is not extended beyond a length of 15 μ , and sometimes not beyond a length of 10 μ . However, where local conditions are less favorable, it pushes out farther (Fig. 17, I, t; Q, t), occasionally attaining a length of 100 μ (Fig. 17, Q). For the most part it varies in width from 2.5 to 5 μ . It is rather markedly expanded at the tip (Fig. 17, G, t; H, t) without, however, sharing the pronounced apical modification characteristic of the evacuation tube in *Pythium salpingophorum*. After discharge of the sporangium its empty membrane in some instances widens noticeably near the orifice (Fig. 17, S, t). Much more often the membrane becomes reflexed at the tip (Fig. 17, H b, t; I-K; t; N-R; t), though such eversion is absent here and there (Fig. 17, L, t; M, t; S, t).

The vesicle attached to the frequently reversed rim of the evacuation

tube is often, especially in its proximal portion, only faintly discernible. In any case the film yields soon after the swarm of active zoospores formed within it begin their battering, whereas in most congeneric species the clearly visible bladder commonly resists the collective impact of the fully fledged swarm for a period of 5 to 8 minutes. Owing in large part to their less prolonged impoundage the zoospores of *Pythium vexans* are often liberated in 13 to 15 minutes after discharge of the sporangium, rather than after the more usual period of approximately 20 minutes. They swim about for some time, then come to rest and round up into subspherical cysts commonly 6.8 to 9 μ in diameter (Fig. 17, T, a-o). They germinate, as a rule, by putting forth a germ tube approximately 1.5 μ wide (Fig. 17, U, a-d).

Besides producing sporangia and zoospores, tracts of young mycelium in slabs excised from maize meal or Lima-bean-agar plate cultures conveniently give rise, on irrigation, to sexual reproductive apparatus in moderate quantity; the softened substratum allowing patently normal development and still retaining enough firmness to hold all imbedded apparatus securely in place for close microscopical examination. As Braun pointed out, pairing of the sex elements takes place at a very early stage. Indeed, even when the young oogonium consists only of a terminal (Fig. 18, A, a) or subterminal (Fig. 18, B, a) enlargement no more than 6 or 7 μ in width, which by itself would not yet be clearly distinguishable from miscellaneous enlargements of vegetative character, it is often found rather extensively in contact with, or extensively enwrapped by a young male complement constituted of a swollen hyphal termination (Fig. 18, B, b) or of such a termination together with a similarly swollen lateral branch (Fig. 18, A, b). Frequently the mycelial connection between oogonial stalk (Fig. 18, A-I: a) and antheridial branch (Fig. 18, A-I: b) is too remote to be traced with certainty amid the confusion of ramifying hyphae. With about equal frequency, however, a connection between the paired elements is plainly evident (Fig. 18, J-O). Sometimes the oogonial stalk (Fig. 18, J, a; K, a) arises from the same hypha as the antheridial branch (Fig. 18, J, b; K, b); sometimes it (Fig. 18, L, a) originates as a secondary ramification from the hypha directly bearing the antheridial stalk (Fig. 18, L, b); or, again, it (Fig. 18, M, a; N, a) provides the very familiar androgynous arrangement of parts in giving rise at a variable distance from the growing oogonium to an antheridial branch (Fig. 18, M, b; N, b) which to reach the place of union follows an arcuate course often considerably rangier (Fig. 18, N, b) than the course of the antheridial branch in monoclinal sexual apparatus in *Pythium debaryanum*. Remoteness with respect to mycelial connection and proximity with respect to position are combined ingeniously in instances where an axial hypha gives off, on the same side, 2 branches (Fig. 18, O, a, b) of which one (Fig. 18, O, a) bears plural oogonia (Fig. 18, O, w, x) that are supplied with antheridia borne on ramifications (Fig. 18, O, y, z) arising from the other (Fig. 18, O, b). In units of sexual apparatus developed directly in maize meal-agar plate cultures prepared with a medium softer

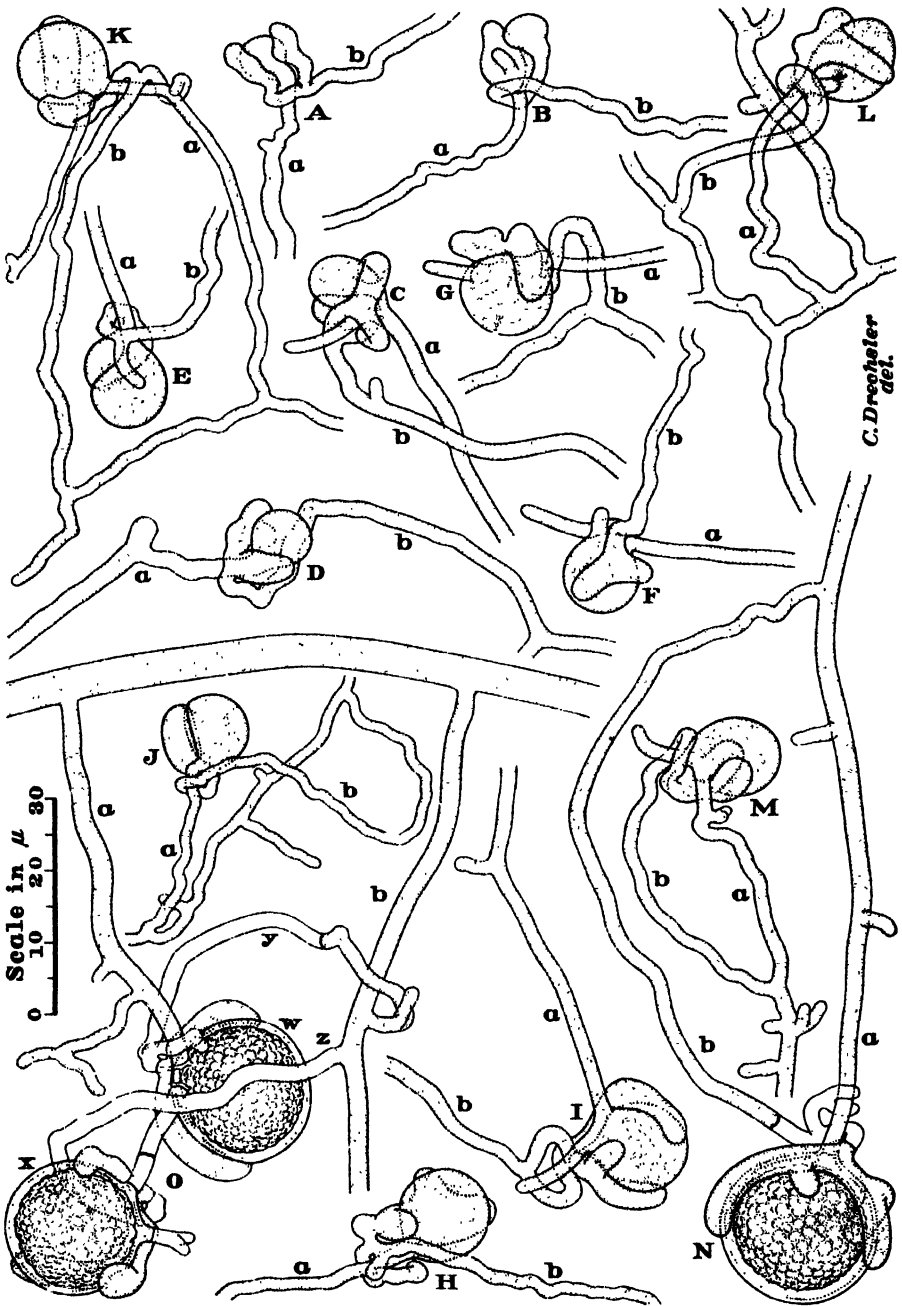


FIG. 18. Immature units of sexual reproductive apparatus of *Pythium vexans* as found produced in soft, irrigated maize meal agar (A-M), and in irrigated Lima-bean agar (N, O); drawn to a uniform magnification with the aid of a camera lucida; $\times 1000$ throughout. A-I. Young units without evident mycelial connection between the paired organs. J-M. Young units each showing moderately close mycelial connection between the apposed organs. N, O. Units about ready for fertilization, each showing a somewhat remote mycelial connection between oogonium and antheridium. (a, hypha supporting oogonium; b, hypha supporting antheridium.)

than the media commonly employed, the mycelial relationships of the conjugating parts differ little from the relationships manifest in irrigated agar slabs. Where a mycelial connection can be made out, the 2 filamentous elements supporting the paired organs here likewise are often contributed by the same hypha (Fig. 19, A, a; Fig. 20, A); or the antheridial branch may arise from the oogonial stalk (Fig. 19, A, b; B-D; Fig. 20, B, C); or secondary and possible tertiary branching may be present in one if not in both of the supporting filamentous elements (Fig. 19, E; Fig. 20, D, E). And, naturally, in soft agar substratum much as in irrigated agar slabs, numerous units of sexual apparatus show no demonstrable mycelial connection (Fig. 19, F 1; Fig. 20, F, G).

As in related species the oogonium develops into a subspherical body. It is often found attached more or less mesially to the end of a hyphal stalk which may be somewhat narrow not only during the earlier formative stages (Fig. 18, A, a; E, a; H, a; J, a) but also during later stages (Fig. 18, N, a; Fig. 19, C, D, F, H; Fig. 20, D), or, again, may be moderately stout (Fig. 18, D, a; Fig. 19, B; Fig. 20, A). A more distinctive hyphal relationship frequently results when the oogonium grows out laterally a short though somewhat variable distance below the tip of the supporting filament (Fig. 18, B, a; C, a; F, a; G, a; I, a; K, a; L, a; M, a), so that a terminal portion of filament, usually about $10\ \mu$ long but occasionally measuring less than $5\ \mu$ (Fig. 18, B, a; F, a; L, a) or more than $25\ \mu$ (Fig. 18, K, a) in length, is borne on the young oogonium after the manner of an appendage. Owing to the circumstance that in soft, yielding substratum the lateral growth of the oogonium often pushes the distal element out of its earlier alignment into a position approximately at a right angle with the supporting element, the origin of the appendage as a termination of the supporting stalk is frequently obscured. When the female organ later comes to be delimited, the distal element is commonly cut off by a cross-wall (Fig. 18, O, x; Fig. 19, A, a, b; I). However, where the distal element is very short, it often remains as a spur-like diverticulum (Fig. 20, D) continuous with the oogonium; this organ thereby being left in a terminal position with a hyphal attachment similar to that of some oogonia having a subspherical shape devoid of marked modification (Fig. 20, G). Sometimes an oogonium develops in laterally intercalary position some distance from the tip of its supporting filament (Fig. 18, O, w). Frequently too, where the oogonium grows out laterally from a base so narrow that the spherical contour does not encroach on the supporting hypha, it becomes delimited as a sessile structure, or is borne terminally on a very short lateral branch (Fig. 19, E, G; Fig. 20, B, C, E, F).

The oogonia of the fungus thus show not only the several relationships to the mycelium that were expressly ascribed by de Bary to the oogonia of *Pythium vexans*, but also the one relationship—attachment to the tip of a slender hypha—which Braun set forth as prevailing in *P. complectens*. Indeed the latter relationship is not wholly unrecorded in de Bary's treat-

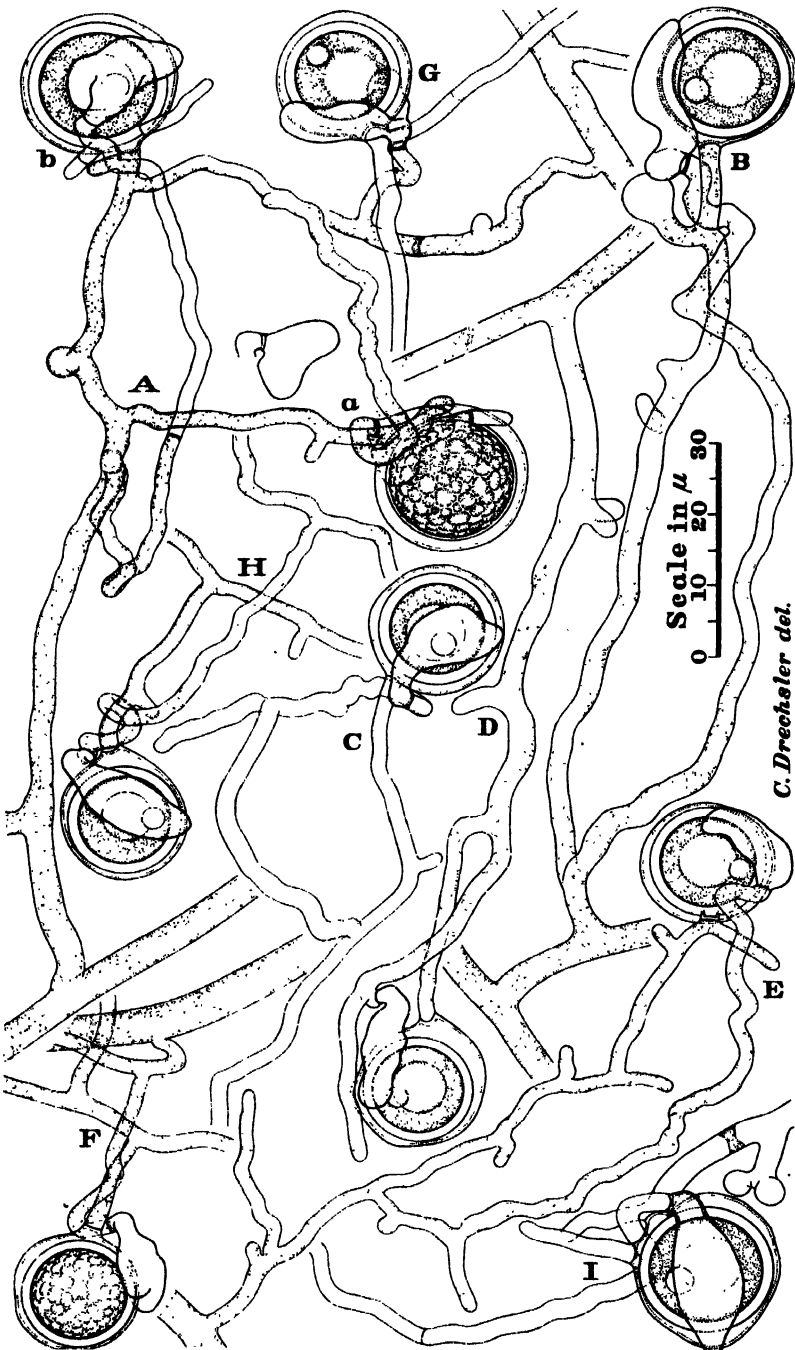


FIG. 19. Sexual reproductive apparatus of *Pythium vexans* drawn with the aid of the camera lucida from Petri-plate cultures prepared from maize meal agar of only moderate firmness; $\times 1000$ throughout. A. Two reproductive units—one (a) with immature oospore, the other (b) with mature oospore—whereof the apposed sex organs are supplied from a single parent filament. B-E. Units of mature apparatus varying with respect to aggregate length of hyphal parts connecting oogonium with antheridium. F.

ment of *P. vexans*; for though not mentioned in his descriptive text, terminal attachment would seem to be represented in one of his figures (4: Taf. V, fig. 4). In my material the supporting hypha has shown no noteworthy tendency to widen markedly below the attachment of a terminally borne oogonium. Such widening appears to have been considered by Braun a feature that de Bary held characteristic of *P. vexans*. Marked hyphal widening immediately below the unipolar attachment of an oogonium borne mesially in alignment with its supporting filament, was figured by Butler as illustrative of the broad insertion of oogonium he deemed especially distinctive of the species. Although de Bary's words describing the oogonium as "theils selbst mit breiter Ansatzstelle eingeschaltet in die Continuität des Schlauches, also, mit andern Worten, intercalar aber einseitig blasig vorgewölbt," unquestionably make reference to broad attachment, the breadth here in question relates to the basal dimension of a laterally intercalary oogonium, and consequently is to be measured lengthwise along, not transversely across, the supporting hypha.

The enwrapment of the young oogonium by the young antheridium, which, as has been mentioned, is observable in irrigated agar preparations at a very early stage, is shared in varying measure by the distal portions of the hyphae bearing the developing sex organs. Often the antheridial branch (Fig. 18, A, b; B, b; E, b; F, b; G, b; L, b) winds half way around the oogonial stalk before extending its widened termination along or about the young oogonium. In the case of subterminal, laterally intercalary oogonia, the distal prolongation rather than the proximal supporting element is often enwrapped by, or interlocked with, the antheridial branch (Fig. 18, C, b; F, b; M, b). Sometimes, again, the antheridial branch passes partly around the young oogonium before it engages with the oogonial stalk and extends its expanded termination over other regions of the globose body (Fig. 18, D, b). The close contact of oogonium and antheridium is maintained as both organs continue growing. While the oogonium merely rounds out into a more nearly spherical shape as it increases in size, the antheridium usually elongates considerably and at the same time often ramifies more or less (Fig. 18, C, b; D, b; F, b; G, b; H, b; I, b; K, b; L, b; M, b). Eventually when the oogonium has attained its full growth, and its readiness for fertilization is made manifest by shrinkage of its lumpy contents from the enveloping wall (Fig. 18, N; O, w, x), it may be found embraced by an antheridium consisting of 2 (Fig. 18, O, w), 3 (Fig. 18, O, x), or 4 (Fig. 18, N) curving finger-like branches measuring individually 5 to 20 μ in length and 2 to 4 μ in width.

Branching of the antheridium is both less frequent and less elaborate in Petri-plate cultures prepared from maize meal agar somewhat firmer than irrigated agar slabs yet not so firm as most gelose media employed in laboratories. Usually in such cultures antheridial branching is represented only

Somewhat immature unit of sexual apparatus without visible mycelial connection between apposed organs. G-I. Mature units of sexual apparatus, each contributed by a pair of hyphal elements without evident mycelial connection.

in simple dichotomy of the ypsiliform (Fig. 19, A, a), bilobate (Fig. 19, F), and biramous (Fig. 19, G) male organs that are observable in moderate numbers. Rather commonly the unbranched male cells here are of elongate saccate shape, perceptibly widened at the middle (Fig. 19, C, D, E, H, I; Fig. 20, B-F). They mostly vary in length from 15 to 30 μ and in width from 5 to 8 μ ; their thickness often being substantially less than their width. Many are applied their entire length to the oogonium; some of the longer ones thus enfolding more than one-third of the circumference of the globose body (Fig. 19, H, I; Fig. 20, B). Contrasting with the numerous antheridia that by their longitudinal application from base to apex recall the homologous organs of my *Pythium helicoides* (20, p. 412-414) and others which because of their application only along their distal half (Fig. 19, D, F; Fig. 20, F) invite comparison rather with the anteriorly applied antheridia occasionally to be observed in my *P. palingenens* (23, p. 491, Fig. 8, C). Somewhat as in irrigated preparations, the antheridial branch in softish agar cultures is often found wound about the oogonial stalk to the extent, as was noted earlier (18, p. 444), of a half turn or whole turn (Fig. 19, A, a, b; B; C; F; H; Fig. 20, B). Even where such involvement is absent, contact of the paired organs or, more especially, contact of their proximal parts, is often accompanied by some more haphazard sort of engagement between the supporting hyphal elements (Fig. 19, G, I; Fig. 20, C, D, E, G); although in other instances no interlocking of the supporting hyphae is evident (Fig. 19, D, E; Fig. 20, A, F).

In irrigated preparations the extensive enwrapment of the oogonium by the antheridium has apparently very little direct effect in modifying the outward form of either organ. Nor is conspicuous modification of shape evident in most units of sexual apparatus produced in rather soft maize meal agar; though here an occasional oogonium may usually be found of which the envelope is broadly indented or flattened in the region of contact with the antheridium (Fig. 19, B; Fig. 20, C), so that it is brought snugly against the oospore wall in the region underlying the antheridium and usually also in the antipodal region. In Petri-plate cultures prepared with maize meal agar of customary firmness, such flattening of the oogonium appears as a virtually constant character, and is commonly associated with malformation of the antheridium often so pronounced that this organ offers an appearance unknown among congeneric species. The scope of deformity displayed in cultures prepared with hard agar is by no means exaggerated in the assortment of misshapen male cells illustrated in Braun's drawings (8: Plate 5, B-D). While conveniently helpful in identifying the species, the bizarre conformation of such antheridia would seem perhaps more nearly a teratological feature than a character pertaining to normal morphology. Owing to the very early apposition of the male and female elements the antheridium in a somewhat unyielding ambient is necessarily subjected throughout the period of its growth to persistent pressure arising especially from the simultaneous expansion of the more massive oogonium; and thus

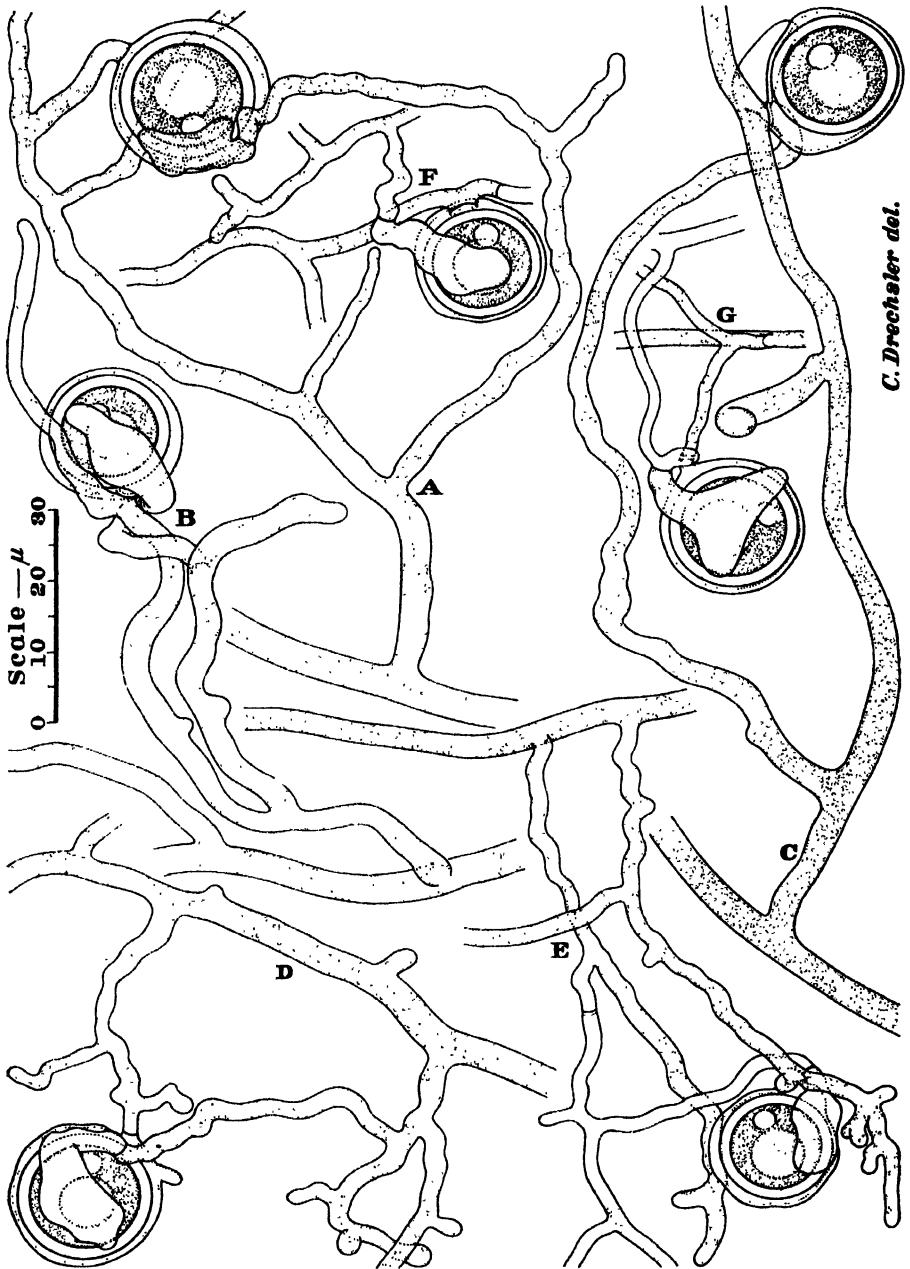


FIG. 20. Mature sexual reproductive apparatus of *Pythium vexans* drawn with the aid of a camera lucida from Petri-plate cultures prepared from maize meal agar of only moderate firmness; $\times 1000$ throughout. A-E. Units of apparatus with obvious mycelial connection between oogonium and antheridium; the antheridium in A, of deep yellow coloration, being applied distally to the oogonium; the antheridium in B-E being empty and colorless and in a deceptive manner having its basal attachment spatially near the base of the oogonium. F, G. Units of sexual apparatus without evident mycelial connection; the empty antheridium in either unit has its base some little distance from the base of the oogonium, but in G a short spur of the supporting branch helps to give somewhat the appearance of close monoclinous relationship.

is squeezed or constrained to grow into a flattened shape fitting the flattened or indented shape of the oogonium. In expanding jointly against the physical resistance of the ambient, the general tendency is for the 2 apposed organs to assume shapes such that the combined unit of sexual apparatus will have a more or less subspherical compact form. As might be expected, the proximal portion of the antheridium is in many instances less affected by this tendency than the median and distal portions.

Although the bilobate ypsiliform antheridium figured by de Bary was mentioned as illustrating a male cell of unusual outward form, it yet provides the most important clue whereby Braun's geranium pathogen can be referred to *Pythium verans*. Among the members of the genus whose oogonia and oospores approach at all closely the measurements for diameter given by de Bary, I have found such bilobate antheridia only in the particular species here under consideration. Bilobate branching has been noted occasionally in *P. helicoides* and in *P. paltingenes* (23, p. 491, Fig. 8, F; p. 492) where the elongated antheridium is similarly applied lengthwise in its extensive enwrapping of the oogonium; but these 2 species, as also the allied *P. oedochilum* Drechsl. (23, p. 478-486), have oogonia and oospores conspicuously larger, not smaller, than those of *P. debaryanum* and *P. ultimum*. The antheridium of semicircular profile shown in one of de Bary's figures (4: Taf. V, Fig. 3) is aptly illustrative of the usual appearance presented by the elongate antheridium of Braun's fungus when it is applied in an equatorial region of the oogonium with its long axis oriented vertically or nearly vertically, that is, in a direction nearly parallel to the line of vision. The very close androgynous relationship figured by de Bary in 2 instances (4: Taf. V, Fig. 3, lower right; Fig. 4), wherein the antheridium is borne on a very short stalk arising from the oogonial hypha in immediate proximity to the oogonium, has not been recognized with certainty in my material. Quite frequently in mature reproductive apparatus the antheridium, because of the position of its basal septum, offered much the appearance of arising in such close monoclinous relationship; but in these instances wherever on careful scrutiny the hyphal connections could be accurately ascertained the male cell was found borne on a separate branch (Fig. 19, A, a, b; B; C; F; H; I; Fig. 20, B-E). Neither has an immediate androgynous relationship been revealed unmistakably in young sexual apparatus (Fig. 18, A-M) where all parts are filled with living protoplasm and thus are most favorable for observation. Later, when the supporting hyphae have been evacuated, their thin, highly transparent membranous envelopes often become so faintly visible as to tax the capabilities of a good modern microscope with good illumination. Since, further, the tubular membranes near the base of the oogonium are often more or less intertwined or interlocked, optical difficulties intrude that would seem well beyond the capacities of the microscopes in use 65 years ago. In fine, regardless of whether the very close monoclinous relationship figured by de Bary is absent in Braun's fungus, or whether it is perhaps occasionally present there, the

very frequent and persuasive simulation of such relationship in my cultures appears under the circumstances to provide sufficient resemblance for identifying the fungus with *P. vexans*.

The passageway through which the antheridial contents migrate into the oogonium is generally even more difficult to see than the hyphae supporting the sex organs. In my material it has been most clearly discernible when observed in profile view in ripened units of sexual apparatus wherein at least locally the oogonial envelope lay in contact with the oospore; the fertilization canal then appearing merely as an aperture, 1.5 to 1.9 μ wide, in the oogonial envelope (Fig. 21, A, B). At maturity, the oospore, commonly 12.5 to 16 μ in diameter and somewhat loosely contained in an oogonial envelope 16 to 21.5 μ in diameter, reveals the unitary organization frequent among members of the genus; its wall, mostly 1 to 1.5 μ thick, surrounding a finely granular layer of protoplasm which encloses a single reserve globule, usually 6 to 9 μ wide, as well as a single globose or slightly flattened refringent body ordinarily 2.6 to 4 μ in diameter (Fig. 19, A, b; B-E; G-I; Fig. 20, A-G). In maize-meal-agar cultures 110 days old fully 9 out of 10 oospores showed no change with respect to internal structure, though some few specimens now revealed 2 refringent bodies. Despite their inert behavior in a stale ambient, the oospores, when transferred to a shallow layer of distilled water, germinated readily by production of zoospores. De Bary recognized the capacity of newly ripened oospores to germinate by the production of swarmers as a characteristic attribute of *Pythium vexans*; and in my material likewise no extended resting period has been required for such development. Like the zoosporangia of mycelial origin among which they developed, oospores taken from maize-meal-agar cultures 40 days old produced swarm spores freely. Fairly abundant development of zoospores ensued also after irrigating oospores removed when the cultures were 110 days old, and again when they were 150 days old.

During the earlier stages of germinative development in an oospore (Fig. 21, A, C-E) the reserve globule changes from a spherical to a somewhat irregular shape, while at the same time the refringent body undergoes division into 4, 5, or 6 bodies appreciably smaller than their parent. Gradually the plural refringent bodies become less clearly recognizable, and before long are lost to view in their granular matrix. The reserve globule also loses some of its distinctness without, however, vanishing from sight. The inner layer of the oospore wall, which as a rule equals or slightly exceeds the outer layer in thickness, takes on more and more the appearance of the protoplasm bordering it, and finally merges indistinguishably with the granular mass. The persistent outer layer then dissolves in a circular area 2.5 to 5 μ wide, permitting the protoplast to protrude against the oogonial envelope (Fig. 21, F). This envelope likewise gives way, and the protrusion emerges externally as a germ tube, except that in occasional instances an overlying antheridium interposes an additional membranous barrier (Fig. 21, G). Often before it has attained a length of 25 μ , the germ tube forms a cap of dehisc-

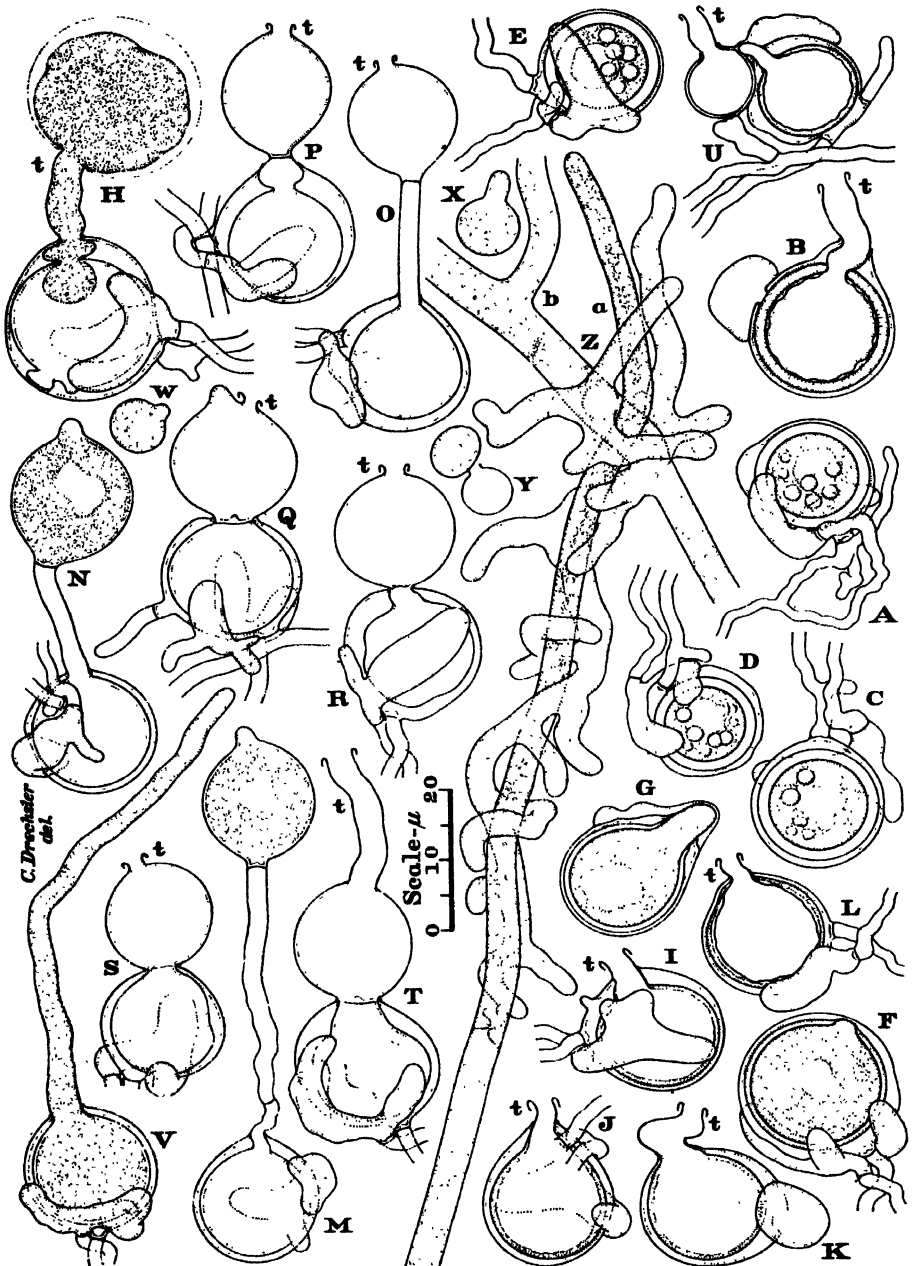


FIG. 21. Drawn with the aid of a camera lucida; $\times 1000$ throughout. A-Y. Germination of oospores of *Pythium vexans* from maize-meat-agar plate cultures 5 months old. A. Oospore showing modification in internal organization preliminary to germination; fertilization passage visible in profile view about 2μ to left of septum delimiting the antheridium. B. Oospore that germinated without reducing the thickness of its wall; a structurally distinct sporangium within the oospore chamber has left its empty envelope continuous with the evacuation tube; showing also the fertilization passage in profile near the middle of the region covered by the antheridium. C-E. Oospores revealing onset of germinative development in irregular outline of reserve globule and in presence of several refringent bodies. F. Oospore whose protoplast has assimilated a thick inner

cence, which yields to permit migration of the protoplasmic materials (Fig. 21, H) into a terminal vesicle where they are fashioned into zoospores. After the zoospores have been liberated the rim of the empty evacuation tube is usually found reflexed (Fig. 21, B, t; I-L: t) much as in sporangia of mycelial origin. Usually when the evacuation tube is followed backward it is found to be continuous with the persistent outer layer of the oospore wall (Fig. 21, I-K). Now and then, however, it appears to be continuous instead with a separate sporangial envelope either nested within the frequently somewhat irregular contour of the yellowish residual layer (Fig. 21, L) or, in the occasional instances where the inner layer has not been digested, nested within the undiminished oospore wall (Fig. 21, B).

Apart from the type of oospore germination wherein the rather broad germ hypha functions directly as an evacuation tube—a type already ascribed to *Pythium vexans* by de Bary—germination in my irrigated preparations has often taken place by the emission from the individual oospore of a somewhat narrower germ hypha that fulfills its function by bearing at its tip a sporangium into which the entire protoplasmic contents are received (Fig. 21, M, N). This sporangium not uncommonly is of citri-form shape, being often provided at the apex with a short protuberance or beak rather suggestive of the prominent papilla familiar especially in certain species of *Phylophthora*, as, for example, *P. cactorum* (Lebert & Cohn) Schroeter. The beak represents an incipient evacuation tube, which frequently, after some slight elongation, yields at the apex to permit the granular contents to migrate into a terminal vesicle for transformation into zoospores; the rim of the empty tubular membrane becoming reflexed (Fig. 21, O, t) in the manner usual for the species. The length of the sporangiferous germ hypha varies usually from 10 to 50 μ (Fig. 21, M-O), yet sometimes it exceeds 100 μ . In many instances, however, similar germinative development takes place without any germ hypha being extended at all; the sporangium (Fig. 21, P-U) here being formed sessile on the oogonium in such wise that after evacuation its membrane is found directly continuous with the residual outer layer of the oospore wall nested within the oogonial envelope, though its basal septum, which usually is found in approximate alignment with the spherical oogonial contour, separates its empty chamber

layer of the wall and has pushed a protrusion through an opening in the outer layer against the oogonial envelope. G. Oospore whose germ tube has broken through the oogonial envelope and is pushing against the farther wall of the overlying antheridium. H. Granular contents of oospore migrating into a vesicle through an evacuation tube conspicuously widened in the space between the oospore wall and the oogonial envelope. I-L. Empty membranous envelopes left behind after escape of swarm spores brought into being following direct conversion of oospore into a zoosporangium. M, N. Oospores, each of which has germinated by producing a sporangium at the tip of a germ hypha. O. Oospore that produced a sporangium on a germ hypha; the sporangium later becoming evacuated in giving rise to zoospores. P-T. Oospores, each of which produced a sporangium sessile on the oogonial envelope; the sporangium then becoming evacuated in giving rise to zoospores. U. Oospore that gave rise within the empty overlying antheridium to a sporangium which subsequently became evacuated in producing swarm spores. V. Oospore with germ hypha apparently of vegetative character. W-Y. Zoospores illustrating stages in the emergence of a secondary motile swimmer. Z. Hypha of *P. vexans*, a, attacked by branches of *Aphanomyces cladogamus* (spinach strain), b. (t, evacuation tube.)

from the equally empty chamber of the oospore. When fully grown such sessile sporangia, like those borne on germ hyphae, are often provided individually with a distal beak, which, again, usually on meager elongation, functions in conveying the protoplasmic contents into a terminal vesicle, and thereafter appears as a short membranous tubulure with reflexed rim (Fig. 21, P, t; R, t; S, t). Sometimes an empty reflexed evacuation tube arising from the distal end of a sporangial envelope measures more than $20\ \mu$ in length (Fig. 21, T, t); wherefore it is evident that the apical beak may elongate rather considerably before serving in discharge of the sporangium. On the other hand, the beak occasionally undergoes no clongation and takes no part in dehiscence; discharge then being effected by means of an evacuation tube having a separate origin (Fig. 21, Q, t). Now and then the germ hypha, after having forced its way through the oogonial envelope or grown through the fertilization canal, enters the empty chamber of the antheridium to produce there a terminal sporangium which consequently has to thrust its evacuation tube (Fig. 21, U, t) through the antheridial membrane to form a vesicle outside. Extension of a germ tube beyond a length of $50\ \mu$ or $75\ \mu$ (Fig. 21, V) often betokens the beginning of mycelial growth and incapacity for immediate development of zoospores. The swarm spores produced in the germination of oospores agree morphologically with those produced from sporangia of mycelial origin; and it seems wholly fortuitous that protrusion of a papilla by encysted zoospores (Fig. 21, W, X) preliminary to emergence of the protoplast (Fig. 21, Y) in the repetitional development of a second swimming generation, has so far come under my observation only in some irrigated preparations containing swarm spores that originated exclusively from oospores.

The frequency of an apiculate shape among sporangia produced from oospores would seem related to their habitually terminal development either on the tip of a germ hypha or directly on the oogonial envelope. Sporangia of similar conformation are found borne terminally also in maize-meal-agar cultures and irrigated preparations but there invite little attention, being often greatly outnumbered by sporangia or conidia of generally subspherical shape that occur in intercalary or subterminal positional relationships. The apiculate sporangia of the fungus contribute to the parallelism with *Pythium helicoides* and *P. palingenae* shown more especially in its frequently elongated clasping antheridia. This parallelism is sustained further in the tendency of the sexual apparatus to take on a peculiar deep yellow coloration distinguishable from the yellowish coloration widely prevalent among species of *Pythium*, not only by its greater intensity but also by its different distribution. For while in *P. vexans*, as in nearly all congeneric forms, the ordinary yellowish coloration is concentrated mainly in the oospore wall, the more unusual coloration most often pervades the interior of the antheridium, giving this organ an appearance as if it were filled with yellow, translucent, homogeneous or faintly granular material (Fig. 20, A), though the presence of a mature oospore of correct internal organization

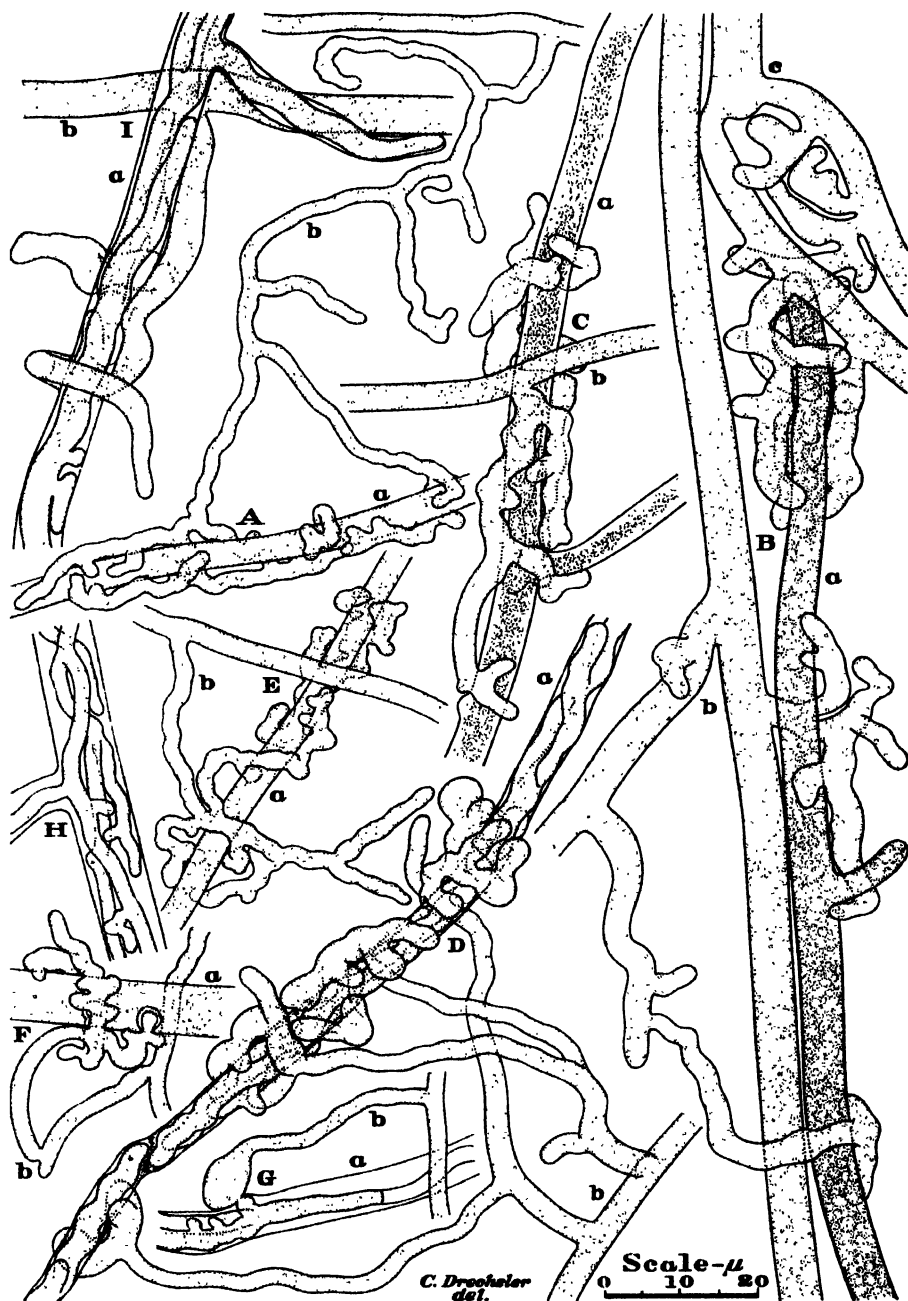


FIG. 22. Antagonistic and parasitic relationships; drawn with the aid of a camera lucida; $\times 1000$ throughout. A. Hypha of *Pythium vexans*, a, attacked by ramifications of *Pythium acanthicum*, b. B. Hypha of *Pythium undulatum* Petersen sensu Dissmann, a, attacked by ramifications from filaments of *Plectospora myriandra*, b and c. C, D. Hyphae of *Pythium undulatum* Petersen sensu Dissmann, a, attacked by ramifications of *Pythium oligandrum*, b. E-H. Hyphae of *Pythium undulatum* Petersen sensu Dissmann, a, attacked by *Pythium acanthicum*, b. I. Hypha of *Pythium undulatum* Petersen sensu Dissmann, a, attacked by *Pythium periplocum*, b.

may give ample proof of effective fertilization. Often the same coloration likewise permeates thoroughly the space between the oogonial envelope and the oospore. In *P. vexans*, as in *P. helicoides* and *P. paltingnes*, such coloration, if of moderate intensity, is not usually concomitant with perceptible abnormality of structure; yet when widespread degeneration of sexual apparatus occurs in these 3 species, it is often accompanied by intense coloration. Whatever the nature of the coloration may be, its development in *P. vexans*, together with resemblances in antheridia and sporangia, suggests that the fungus may perhaps be somewhat more closely related to the *helicoides* series than most of the numerous congeneric forms similarly having nonproliferous sporangia and oospores of unitary internal organization.

The species, as was noted, has been isolated from discolored tomato rootlets, and thus is known to occur on the same host as the saprolegniaceous form I have described as *Plectospora myriandra* (15). When it is grown in maize-meal-agar plate cultures in opposition to that water mold its advance is halted abruptly at the line of encounter, its individual hyphae (Fig. 16, C, a) being made to degenerate internally soon after they have become elaborately invested by short branches extended from the main filaments of the opponent mycelium (Fig. 16, C, b). Likewise when it is grown in opposition to the saprolegniaceous root-rot fungus *Aphanomyces cladogamus*, with which it shares common host relationships through its known occurrence in roots of tomatoes, spinach, and pansies, its mycelial advance is abruptly halted at the line of encounter, and its hyphae at the forefront (Fig. 21, Z, a) suffer visible degeneration promptly after they have been enwrapped by elaborately ramifying branches from filaments of the water mold (Fig. 21, Z, b). Growing in the presence of *Pythium periplocum* the species often shows markedly varied behavior in different portions of the same Petri-plate culture. In some regions many of its hyphae (Fig. 16, D, a) may become extensively if somewhat loosely invested by irregular branches arising from the filaments (Fig. 16, D, b) of the spiny form, and then suffer invasion by assimilative elements intruded into them. In other regions its hyphae not only remain wholly unharmed but are found bearing rather massive appressoria (Fig. 16, E, a; F, a), each of them affixed apically to a *periplocum* filament (Fig. 16, E, b; F, b); a short, frequently lobate protrusion which extends from the tip of the appressorium into a thick deposit of golden yellow substance within the filament indicating that invasion was stopped by secretion of a defensive barrier. When the species is grown in Petri-plate cultures in opposition to *Pythium acanthicum*, many of its hyphae (Fig. 16, G, a; Fig. 22, A, a) along the zone of encounter become enveloped by intricately ramifying branches of the echinulate fungus (Fig. 16, G, b; Fig. 22, A, b). The injury sustained appears usually not very serious, for although some of the invested filaments suffer internal degeneration while others in addition are invaded by assimilative elements, envelopment in numerous instances seems not to result in any abnormal changes.

PYTHIUM ANANDRUM

Since the descriptive account (20, p. 415-420) supplementary to the original diagnosis (17, p. 410-411) of *Pythium anandrum* was written, the fungus has been isolated by Hickman (28) from strawberry (*Fragaria* sp.) roots received from Scotland, and, besides, has been made known by Middleton (34) as occurring in the United States on cucumber fruits, bean roots, and spinach roots. In view of the wider host range and more extensive geographical distribution thus disclosed, it is of moment that the main difficulty hitherto experienced in trustworthy identification of the fungus—the difficulty of obtaining the papillate zoosporangia distinctive of the species through irrigation of young mycelium—can be circumvented advantageously, if the occasion is not too pressing, by using, instead of young mycelium, the parthenospores always abundantly formed in maize-meal-agar cultures. Structurally separate zoosporangia of the sort most helpful in making determinations are commonly formed in ample quantity by germinating parthenospores even though many parthenospores dispense with the development of such bodies in giving rise to swarmers. The behavior of the fungus in the laboratory suggests that under natural conditions *P. anandrum* may very probably produce its zoospores in larger measure in the germination of its parthenospores than from its sporangia of mycelial origin.

On transfer to a shallow layer of water, parthenospores of *Pythium anandrum* taken from a maize-meal-agar plate culture 11 days after planting, that is, only a few days after they had achieved the unitary internal organization of maturity, showed germination in scattered instances. When the cultures were 90 days old, nearly all of the parthenospores germinated on similar treatment, with few exceptions giving rise to swarm spores. In the earliest recognizable stage of germinative development, 2 (Fig. 23, A) to 4 (Fig. 24, A) refringent bodies, evidently derived by division of the single refringent body present earlier, may be seen in the finely granular parietal layer of protoplasm. The reserve globule, which during the resting period has an accurately spherical boundary, now shows a noticeably irregular contour; and the inner layer of the oospore wall, embracing about two-thirds of the thickness of this envelope, reveals closely arranged radial linear markings (Fig. 23, A). Through further change the inner layer of the wall gradually becomes indistinguishable from the granular layer in contact with it (Fig. 23, B), and soon its substance amalgamates with the protoplast, which thus becomes expanded to reach the persistent outer layer of the wall. This outer layer dissolves in a round area 2.5 to 8 μ wide, permitting the protoplast to protrude against the oogonial envelope. When the oogonial envelope likewise gives way locally, the protrusion pushes out as a germ hypha (Fig. 23, C, t; Fig. 24, B, t; C, t). Germ tubes destined to function directly as evacuation tubes grow out, often with abrupt changes in direction, to a length of 10 to 65 μ and at a width varying commonly from 4.5 to 6.5 μ (Fig. 23, D-G; t; Fig. 24, D, t; E, t), though here and there some may expand locally to a width of approximately 10 μ (Fig. 23, G, t; Fig.

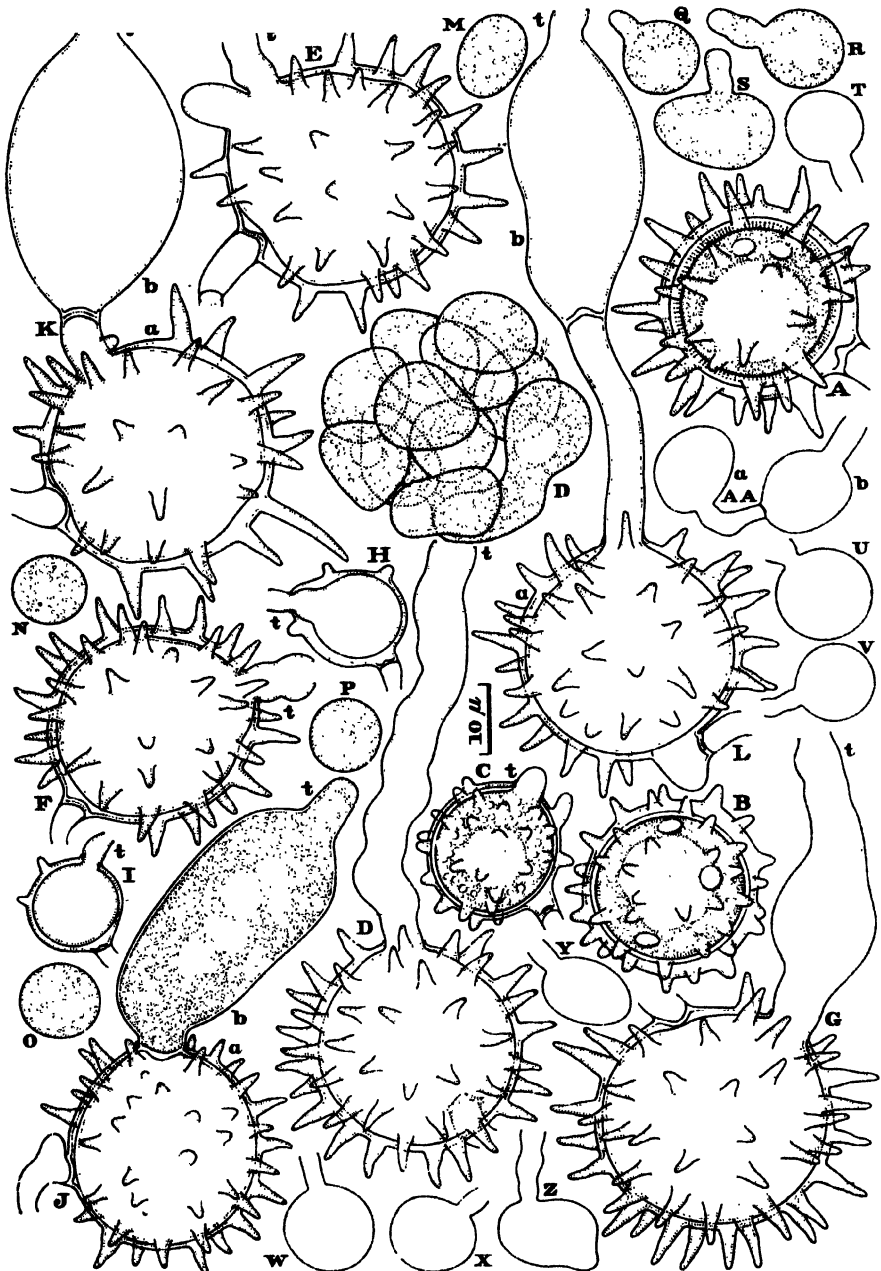


FIG. 23. Germination of parthenospores of *Pythium anandrum* from maize meal-agar cultures 3 months old; drawn with the aid of a camera lucida; $\times 1000$. A, B. Parthenospores showing onset of germinative development in assimilation by protoplast of inner layer of wall, in irregular outline of reserve globule, and in presence of plural refringent bodies. C. Parthenospore that has resorbed inner layer of wall and extended a germ tube through oogonial envelope. D. Parthenospore that has discharged its contents by way of a long evacuation tube into a vesicle which has disappeared; the resulting zoospores having encysted in place. E-J. Membranous envelopes left behind after escape of the zoospores formed through conversion of parthenospores directly into sporangia. J. Parthenospore, a, that has produced a sporangium, b, sessile on the oogonial envelope. K, L.

24, E, t). A very small parthenospore measuring approximately 12μ in diameter may produce an evacuation tube scarcely 5μ long and 3μ wide (Fig. 23, H, t; I, t). In the vesicle formed apically when the protoplasmic contents of so small an oospore flow through the minute evacuation tube only 2 zoospores are fashioned. The granular materials from an oospore 27μ in diameter (Fig. 23, D) are sufficient for about 12 swarmers. As many as 15 or 16 zoospores have been produced in the largest parthenospores, which measure 29μ or 30μ in mean diameter (Fig. 23, G; Fig. 24, E). Individualization of the motile spores always takes place within a vesicle after the manner usual in the genus. If from lack of water the vesicle disintegrates somewhat prematurely the developing zoospores often encyst in irregular shapes to form a cluster near the open end of the evacuation tube (Fig. 23, D).

The parthenospores of *Pythium anandrum*, like the homologous reproductive bodies of *P. salpingophorum* and *P. vexans*, give rise to swarmers not only by becoming directly transformed into zoosporangia, but also, as has been intimated, by producing sporangia structurally distinct from themselves. In instances of the latter type of germinative development the germ hypha may attain a length exceeding 200μ before its tip begins to expand in initiating the formation of a terminal apically papillate elongated-ellipsoidal sporangium which receives all or very nearly all the protoplasmic content of the parthenospore before it is delimited by a basal septum. Where the layer of water is kept shallow, so that deep immersion is avoided, the sporangiferous germ hyphae only occasionally will exceed 100μ in length (Fig. 24, F), and most often will measure less than 75μ in this dimension (Fig. 24, G). They commonly vary in width from 3 to 5.5μ (Fig. 23, K, L; Fig. 24, F, G) and thus are appreciably narrower, besides being less irregular in course, than germ hyphae destined to operate as evacuation tubes. In meagerly irrigated preparations a special sporangiferous hypha is frequently dispensed with altogether, as the germ tube here often widens immediately after pushing through the oogonial envelope (Fig. 23, J, a) and forms a sessile sporangium (Fig. 23, J, b) delimited at the base by a septum flush with the parthenospore membrane. A sporangiferous hypha may not be formed even where the germ tube elongates as a stout filament for some distance outside of the oogonial envelope, since the basal septum delimiting the sporangium is sometimes laid down as a broad convex partition within the chamber of the parthenospore (Fig. 24, H). At times a germ tube that elongated externally as a filament only for a few microns may nevertheless furnish a recognizable, if short, supporting stalk (Fig.

Parthenospores, a, of which each produced a sporangium, b, at the end of a germ hypha; the sporangia later becoming evacuated in giving rise to swarm spores. M-P. Encysted zoospores. Q-S. Encysted zoospores, showing different stages in production of an evacuation tube for emission of a secondary motile swarmspore. T-Z. Empty cyst envelopes left behind after escape of a secondary motile zoospore from each. AA. Membranous envelopes evidencing production by encysted zoospore, a, of a minute zoosporangium, b, at the tip of a germ tube; the sporangium then having produced a secondary motile swarm spore. (t, evacuation tube.)

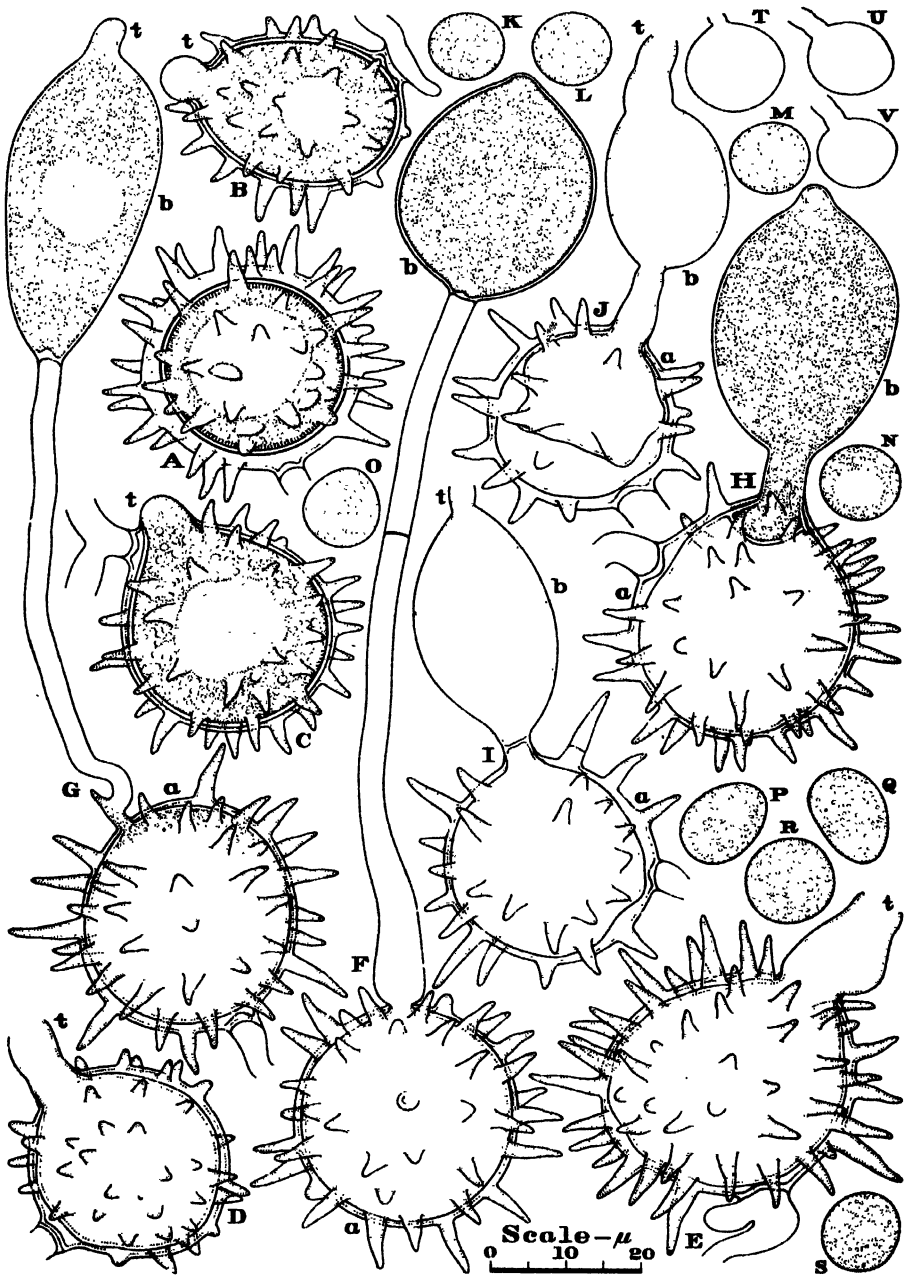


FIG. 24. Germination of parthenospores of *Pythium anandrum* from maize-meal-agar plate cultures 3 months old; drawn with the aid of a camera lucida; $\times 1000$. A. Parthenospore showing onset of germinative development in assimilation by the protoplast of inner layer of wall, in irregular outline of reserve globule, and in presence of plural refringent bodies. B, C. Parthenospores that have resorbed an inner layer of wall and extended a germ tube through oogonial envelope. D, E. Membranous envelopes left behind after escape of zoospores formed through conversion of parthenospores directly into sporangia. F, G. Parthenospores, a, each of which has produced a sporangium, b, at the end of a germ hypha; the germ hypha in G emerging from one of the oogonial spines. H. Partheno-

24, I); whereas at other times a germ tube that elongated externally as a filament for fully $10\ \mu$ before widening may come to constitute the constricted median portion of a dumbbell-shaped sporangium one of whose expanded parts is deeply nested within the chamber of the parthenospore (Fig. 24, J, a), while the other occupies a position corresponding to the usual position of a stalked sporangium (Fig. 24, J, b). In instances of such partly endogenous origin of the germ sporangium, the obvious separateness of the sporangial wall from the parthenospore membrane suggests that where the empty evacuation tube in *P. vexans* is found continuous with a separate membranous envelope deeply inserted into the chamber of the oospore (Fig. 21, B, L), formation of swarm spores came about by development of a wholly endogenous sporangium rather than through direct conversion of the oospore into a sporangium.

Germ sporangia, when borne terminally on germ hyphae (Fig. 24, F, b; G, b) or sessile on oogonia (Fig. 23, J, b), correspond well in their generally ellipsoidal and distally papillate shape to the sporangia of mycelial origin that were described earlier. While the apical papilla here likewise often forms a cap of dehiscence directly, so that the vesicle into which the protoplasmic materials migrate is often sessile on the sporangium, numerous instances came to light in which the papilla elongated materially before discharge took place (Fig. 23, J, t; Fig. 24, G, t). Consequently the empty sporangial envelope was often found extended distally into an evacuation tube varying mostly from 1 to $10\ \mu$ in length (Fig. 23, K, t; L, t; Fig. 24, I, t; J, t). Sometimes, though less frequently than in *Pythium vexans*, the evacuation tube was found in a position apart from the apical papilla. Proliferous development of sporangia, such as takes place rather sparingly when young mycelium in agar slabs is irrigated, has never been observed in the germination of parthenospores. Lack of renewed sporangial growth here has sufficient explanation in the limited volume of the parthenospore; for even when a relatively large specimen contributes its entire contents, the resulting sporangium is yet substantially smaller than the average sporangium of mycelial origin. Nor have parthenospores ever been observed giving rise to plural sporangia on separate germ hyphae.

It is not evident that the zoospores brought into being through germination of parthenospores differ from those produced after appropriate irrigation of young mycelium. In a random assortment of encysted individuals (Fig. 23, M-P; Fig. 24, K-S) some may usually be found to measure only $10\ \mu$ in diameter (Fig. 23, N, P; Fig. 24, K). Smaller size of cysts is sometimes partly attributable to prevalence of repetitional development. Most frequently such development is initiated by the cyst through extension of an

spore, a, that has produced a sporangium, b, partly inserted into the chamber of the parthenospore envelope. I. Parthenospore, a, that has produced a sporangium, b, on a very short stalk; the sporangium later having become evacuated in giving rise to swarm spores. J. Parthenospore, a, that produced a sporangium, b, with a basal part deeply nested within the parthenospore envelope; the whole sporangium having become evacuated in giving rise to zoospores. K-S. Encysted zoospores. T-V. Empty cyst envelopes left behind after escape of a motile secondary zoospore from each. (t, evacuation tube.)

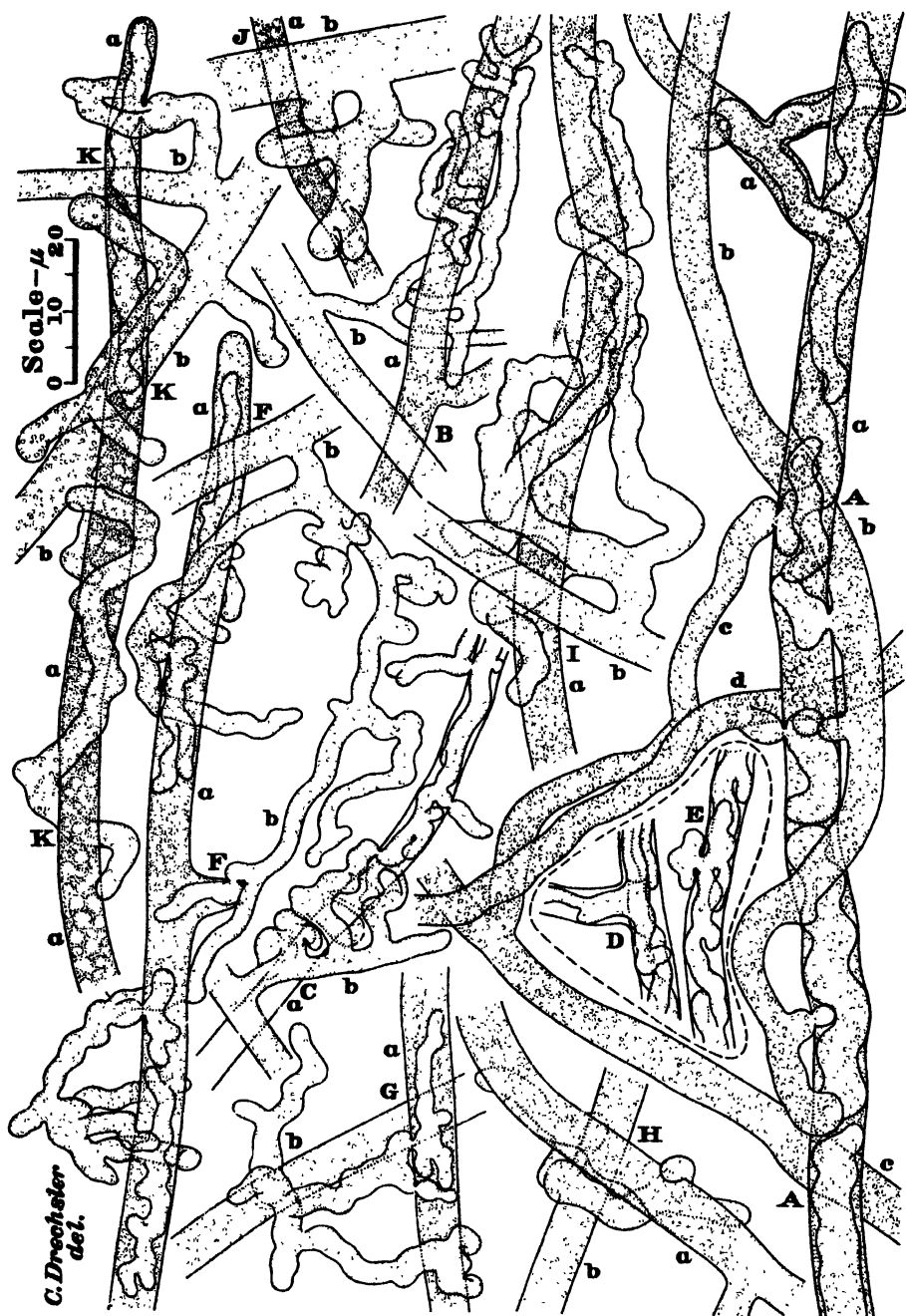


FIG. 25. *Pythium anandrum* attacked by other oomycetes; drawn with the aid of a camera lucida; $\times 1000$ throughout. A. *Pythium anandrum*, a, attacked by *Pythium oligandrum*, b, c, d. B, C. *Pythium anandrum*, a, attacked by *Pythium acanthicum*, b. D, E. Hyphae of *Pythium anandrum* invaded by *Pythium acanthicum*. F, G. *Pythium anandrum*, a, attacked by *Pythium periplocum*, b. H, I. *Pythium anandrum*, a, attacked by *Pleotospira myriandra*, b. J, K. *Pythium anandrum*, a, attacked by *Aphanomyces cladogamus* (pansy strain), b.

evacuation tube (Fig. 23, Q-S) 1.8 to 10 μ long and 2 to 4 μ wide. The empty cyst envelope left after the secondary zoospore escapes from the small terminal vesicle shows considerable variation with respect to the length, width, and shape of the open membranous tube (Fig. 23, T-Z; Fig. 24, T-V). Empty membranous envelopes (Fig. 23, AA) were occasionally observed that supplied evidence of less direct repetitional development; the primary zoospore (Fig. 23, AA, a) in each instance having manifestly given rise on the tip of a germ tube to a small sporangium (Fig. 23, AA, b) which then extended an evacuation tube to permit escape of the contents into a terminal vesicle for transformation into a secondary swarmer.

When *Pythium anandrum* is grown in maize-meal-agar plate cultures in opposition to *P. oligandrum*, its mycelial advance is halted at the zone of encounter, and along this zone many of its hyphae become loosely enwrapped with hyphae and branches of the more delicate spiny form. Such enwrapment is usually soon followed by visible degeneration of protoplasmic contents. Some of the invested filaments (Fig. 25, A, a) further undergo invasion lengthwise by numerous assimilative elements intruded here and there by branches and diverticula arising from *oligandrum* filaments (Fig. 25, A, b-d). When the fungus is grown in opposition to *P. ucanthicum* its mycelium is likewise halted at the zone of encounter; its hyphae at the forefront (Fig. 25, B, a; C, a) again becoming abundantly enveloped by intricately ramifying branches of the less robust echinulate species (Fig. 25, B, b; C, b). Many of the enveloped hyphae rupture near the tip, releasing considerable quantities of protoplasmic material. In any case the hyphal contents soon take on the somewhat opaque appearance associated with degeneration (Fig. 25, B, a). Often, besides, the enveloped hyphae are invaded by irregularly ramifying assimilative elements (Fig. 25, C, a; D; E) which after appropriating the granular materials will frequently push out through the confining membrane to extend the infection, if possible, to neighboring hyphae. Similar injury is sustained by *P. anandrum* when it is grown in opposition to *P. periplocum*. Along the zone of encounter its hyphae (Fig. 25, F, a; G, a) are promptly arrested in their growth as they are beset by intricate ramifications extended from the *periplocum* filaments. The hyphae attacked soon take on a darkish appearance; their degenerating protoplasm in many instances being appropriated by assimilative branches invading them longitudinally (Fig. 25, F, G). When *P. anandrum* is grown in opposition to *Plectospora myriandra*, its mycelial advance, again, is abruptly halted at the zone of encounter. Its hyphae (Fig. 25, H, a; I, a) at the forefront become enveloped by ramifications put forth from filaments of the saprolegniaceous fungus (Fig. 25, H, b; I, b). Such envelopment is regularly followed by internal degeneration, and often also by invasion with assimilative branches. Under similar conditions the related *Aphanomyces cladogamus* likewise arrests the mycelial advance of *P. anandrum* at the zone of encounter; many of the *Pythium* hyphae (Fig. 25, J, a; K, a) soon becoming enwrapped by branches extending from *Aphanomyces* fila-

ments (Fig. 25, J, b; K, b). The enwrapped portions of hyphae degenerate internally, and frequently, moreover, undergo invasion by assimilative branches of the saprolegniaceous species.

PYTHIUM UNDULATUM PETERSEN SENSU DISSMANN

In the well-known account of Danish fresh-water phycomycetes published by Petersen in 1909 (37) and again in 1910 (38) this author describes as a new species under the binomial *Pythium undulatum* a fungus he had found living especially on the leaves and petioles of both the white waterlily, *Nymphaea alba* L., and the European yellow pondlily, *Nuphar luteum* (L.) Sibth. and Smith, though occasionally occurring also on the buds of these aquatic phanerogams and on old fruits of iris as well as on branches of trees. Its extramatrical mycelium was set forth as consisting of unbranched, more or less undulating hyphae, often several millimeters long and 3 to 6 μ wide. To the species were ascribed terminal (rarely lateral) ellipsoidal sporangia, about 130 μ long and 50 μ wide, which sometimes were provided with a small apical papilla. The sporangia were stated to open at the apex, sometimes with a papilla; no explanation being given, however, as to whether the papilla was always operative in dehiscence when it was present, or by what means dehiscence was accomplished when it was absent. Laterally biciliate zoospores similar to those of *Pythium*, it was asserted, issued forth, measuring 15 to 20 μ in length presumably while in their motile condition rather than after their encystment. Despite the phrase "in vesica ut in Pythio," the sequence in which the descriptive details are given leaves uncertainty, as Blackwell, Waterhouse, and Thompson (7, p. 154) have justly intimated, whether the zoospores are really fashioned within a *Pythium* vesicle, or whether, as sometimes happens among species of *Phytophthora*, they are surrounded for a very short time in a highly evanescent vesicle after being discharged from the sporangium in a full-fledged state. After escape of the zoospores new sporangia are sometimes formed within the old ones, while at other times the supporting hypha produces a sporangium after growing lengthwise through the empty envelope. The protoplasm was characterized as refractive, and the membranes of the hyphae as more or less brownish. Of the several sporangia shown in Petersen's 4 relevant drawings, 2 are filled with contents and show an apical protuberance that might represent either a very prominent sessile papilla or a short evacuation tube (37: Fig. VIII, a (right); d); one of these two being borne at the tip of a hypha in a group with 2 empty sporangial envelopes (37: Fig. VIII, a, top, left) which reveal distally a recognizably narrowed prolongation of the membrane as if a short evacuation tube, not a sessile papilla, had been operative in discharge. However, no similar narrowed prolongation is evident in the illustrations of 2 other sporangial envelopes (37: Fig. IX), neither of which assuredly could at any stage have been continuous with an evacuation tube. From the scale of magnification indicated for them the 2 distally unmodified empty envelopes would seem to have dimen-

sions most extraordinary for sporangia of such ellipsoidal type; the larger one appearing to measure about $240\ \mu$ in length, $70\ \mu$ in greatest transverse diameter, and $28\ \mu$ in width of apical opening.

Even if these extraordinary dimensional values are disregarded, so that only the measurements given in the diagnosis are left for consideration, the large size of the ellipsoidal sporangia appears to be the most distinctive character presented in the original account of *Pythium undulatum*. Despite wide variability in size resulting from differences in environal conditions, ellipsoidal sporangia $130\ \mu$ long and $50\ \mu$ wide are not often encountered among species of *Pythium*. In most representatives of the genus—certainly in most of those commonly found in a terrestrial habitat—the tendency toward moderation in volume of the sporangium appears fairly pronounced; somewhat extensive swollen branching systems in luxuriant irrigated material of *P. Butleri*, for example, often becoming divided by a dozen cross-walls to no other end, apparently, than to lessen the size of the individual reproductive units. It is true, Apinis (1) ascribed elongated oval sporangia, 50 to $167\ \mu$ long and 20 to $50\ \mu$ wide, to a submersed fungus that he held referable to Petersen's species; but he transferred this species to *Pythiomorpha* presumably because in his material the zoospores were formed directly within the sporangia. Matthews (32, p. 69–71) somewhat doubtfully recognized *Pythium undulatum* in a fungus isolated from soil in North Carolina which when grown on hemp seeds in distilled water produces proliferous cylindrical sporangia, 25 to $55\ \mu$ long and 15 to $18\ \mu$ wide, that evidently discharge their contents into a vesicle for transformation into zoospores. Narrowly ovoid proliferous sporangia, likewise functional in asexual reproduction typical of *Pythium*, but of even smaller size than those of Matthews—their length usually varying from 40 to $45\ \mu$, and their width from 12 to $15\ \mu$ —were produced by an aquatic fungus that Sparrow (40, p. 299–300) discussed at first hand under the binomial contributed by Petersen. Working in Denmark a quarter of a century after Petersen, Lund (31, p. 48–51) applied the binomials *Pythiomorpha undulata* and *Pythium undulatum* to things he regarded as separate: Apinis' species being recognized in a fungus which he isolated from various natural substrata—plant remains, *Sphagnum*, soil, sand, twigs (including *Picea* twigs), roots of *Salix repens* L.—and which formed zoospores directly within terminal sporangia usually oval in shape, 45 to $117\ \mu$ long, 35 to $43\ \mu$ wide, frequently $70\ \mu$ long and $40\ \mu$ wide; while Petersen's species was doubtfully recognized once in a sporangium of similar morphology that bore at its mouth a vesicle in which zoospores were lying. Despite the meagerness of the material that provided Lund's momentary experience ostensibly with *Pythium undulatum*, his view that members of 2 different genera have come to share the specific epithet *undulatum* given by Petersen seems amply justified. This view is reflected in Sparrow's (41, p. 707) recent treatment of *Pythiomorpha undulata* as a phycomycete distinct from *Pythium undulatum*; the widely divergent usage with respect to the epithet having somewhat earlier been reviewed by Blackwell, Waterhouse,

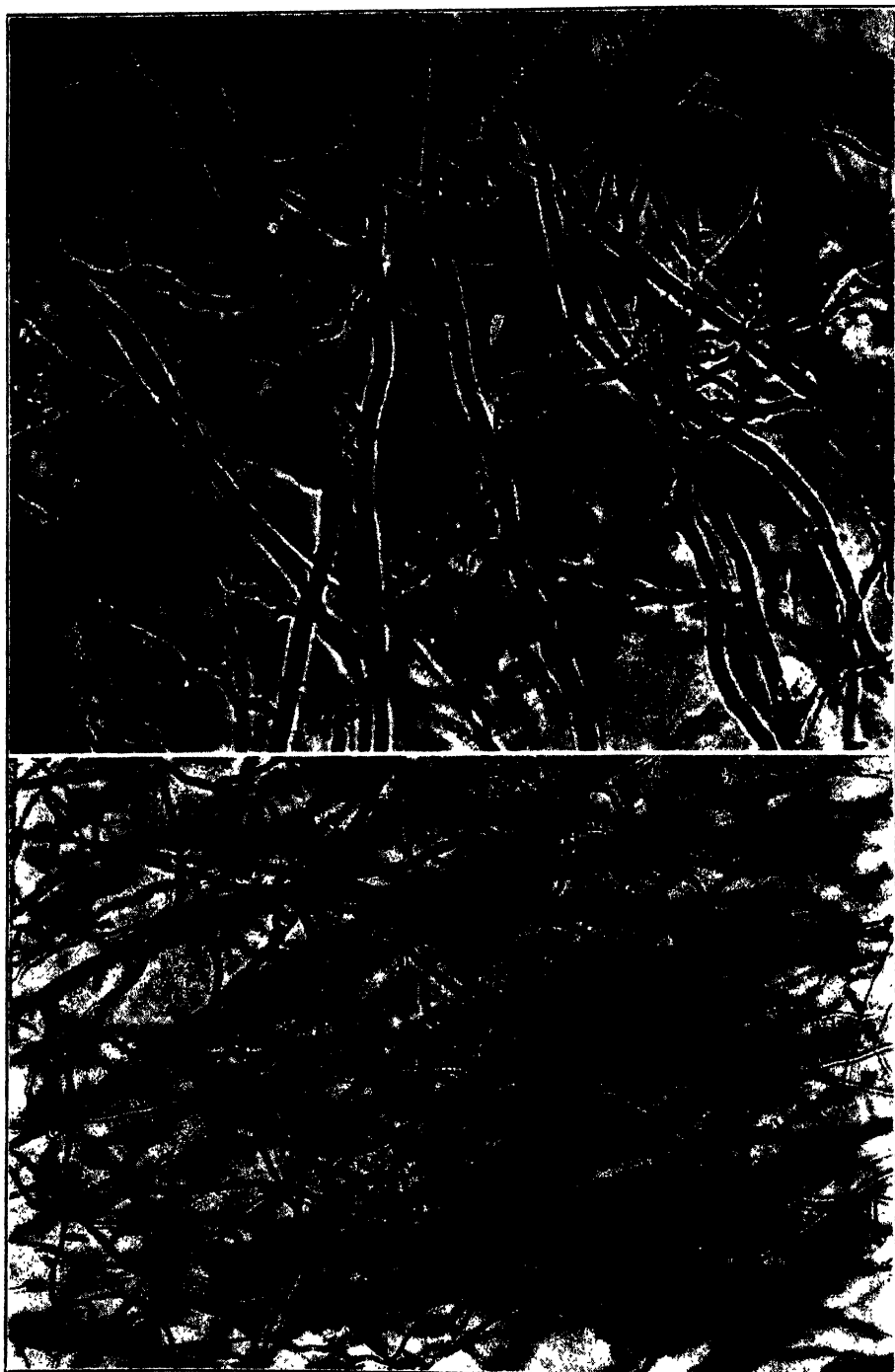


FIG. 26. Mycelium of *Pythium undulatum* Petersen *sensu* Dissmann as found in a 2-day-old maize meal-agar plate culture; photomicrographs; magnification in A about $\times 240$, in B about $\times 120$.

and Thompson (7) in their elucidation of the evident synonymy of *Pythiomorpha* (including *Pythiomorpha undulata* as understood by Apinis and Lund) with *Phytophthora*.

If ellipsoidal proliferous sporangia approximately of the extraordinary dimensions given in the original diagnosis of *Pythium undulatum* were to be found usual and characteristic for some one particular species—preferably for a species in which undifferentiated protoplasmic contents are delivered into a vesicle and then are fashioned into motile zoospores—no serious misgivings could be entertained with respect to the correct application of the binomial. Although the relevant literature does not offer any completely satisfactory metric agreement, a rather likely application of the binomial is given in Dissmann's (13) account of 2 *Pythium* species he isolated from prematurely discolored, yellowing leaves of waterlily (*Nymphaea candida* Presl.) plants that grew abundantly in an artificial pond near Hirschberg in Bohemia; both species, therefore, being similar to Petersen's in regard to their host relationship and their aquatic habitat. In peptone-saccharose solution the fungus Dissmann identified as *Pythium undulatum* produced a mycelium with hyphae up to 6 or 8 μ in width; in pea decoction its mycelium branched more irregularly and grew more delicately by extending hyphae often only 3 to 4 μ wide. The sporangia obtained by transferring mycelium from a liquid culture to water varied greatly in size with changes in the concentration of the nutrient solutions employed: a solution containing from 0.5 to 1.0 per cent of peptone yielded sporangia 30 to 40 μ long, whereas a 1.0 per cent solution yielded sporangia 40 to 76 μ in length. Use of a 0.6 per cent haemoglobin solution resulted in production of sporangia up to 60 μ long. When the fungus was transferred to unmodified pond water after being grown in pond water containing 5 per cent of maltose it produced sporangia up to 80 μ long. On transfer to unmodified pond water, mycelium that was grown in pond water fortified with waterlily-leaf decoction gave rise to very large sporangia, with an occasional individual measuring up to 120 μ or 140 μ in length—dimensional values close to those found in material developed under natural conditions. In a figure (13: Fig. 1) showing zoosporangia drawn as they were found occurring naturally in a mixture on waterlily leaves, those ascribed to *Pythium undulatum* (13: Fig. 1, a)—all represented by empty envelopes borne on a single ramified trunk—appear from the scale of magnification to range in length from 60 to 150 μ . None of these envelopes shows any narrow prolongation at the open distal end. Their general appearance suggests that discharge must have been accomplished by means of a sessile cap of dehiscence rather than by means of an evacuation tube. In the multiple nesting of empty sporangia illustrated in the figure some of the inner envelopes terminate so far within the outer envelope that formation of a globose vesicle would have been obstructed. Among proliferous species of *Pythium* such obstruction does not ordinarily occur, since here as a rule the inner sporangium is extended either broadly or by an evacuation tube until its tip is flush with,

or protrudes beyond the mouth of the older membranous mantle. Instances of recessed nesting are, however, not infrequent among proliferous species of *Phytophthora*, where no vesicle needs to be formed, and where the full-fledged zoospores released from an inner sporangium are capable of making their way if necessary through a series of apical openings. The generous width of the apical openings, which here and there would seem to exceed $15\ \mu$, is likewise especially suggestive of *Phytophthora*, in which the non-papillate sporangia of various proliferous species, as, for example, *P. cryptogea* Pethyb. & Laff. and *P. cambirora* (Petri) Buisman, are given to dehiscence by a relatively broad distal aperture. In contrast, both of the correctly evacuated zoosporangial envelopes (13: Fig. 8, left; Fig. 9) drawn by Dissmann from material derived from the pure culture that he treated as *Pythium undulatum* show a recognizable evacuation tube at the apex; the wider of the 2 tubes measuring about $9\ \mu$ in diameter. There is good reason to suspect that the strongly proliferous sporangia drawn from waterlily material originating in nature were alien to the fungus represented in the pure culture—that they belonged more probably to a species of *Phytophthora*, which, owing perhaps to the slower mycelial extension usual in members of this genus, may have been consistently outgrown in isolation cultures by the accompanying species of *Pythium* and thus kept from being recognized.

Apart from sporangia, Dissmann's pure culture of *Pythium undulatum* readily gave rise on various agar substrata to terminal or intercalary subspherical reproductive bodies he termed chlamydospores; the protoplasm for their growth being obtained through a progressive evacuation of adjacent portions of hypha entailing deposition of successive boundary walls. The size of the chlamydospores was found to increase with the richness of the agar medium employed. In maize-meal-agar cultures prepared with media whose nutrient concentration varied in a 1–5–10 ratio, the most frequent values for diameter of the globose spores were about $36\ \mu$, $59\ \mu$, and $69\ \mu$, respectively; a total range extending from $6\ \mu$ to $92\ \mu$ being indicated for the dimension. Rather early in the development of the chlamydospore its wall could be recognized as composed of 2 layers, and with advancing maturity the two-layered construction became more distinctly visible: the very thin outer layer consisted of the original hyphal envelope, while the inner layer, of more variable thickness, represented a special membrane secreted by the protoplast. In chlamydospores older than those present in maize-meal-agar cultures 4 weeks after inoculation, the outer layer was stated to persist as flakes or lumps externally adnate to the inner layer. With respect to the internal organization of the chlamydospore, aging was found accompanied by conspicuous accumulation of fat (Fett); this material first appearing in small droplets, and later, following union of the small droplets, in larger globules. Attempts at germinating the chlamydospores were successful only with very young specimens in which no accumulation of fat, nor any thickening of the wall had yet taken place. In such young

specimens the thin envelope ruptured, or a short evacuation tube was put forth; the protoplasmic contents, in either event, being then emptied into a vesicle for transformation into zoospores. Failure of the older chlamydospores to germinate, it was pointed out, might derive from conditions similar to those present in oospores, which likewise constitute a thick-walled resting stage with abundant accumulation of fat, and which likewise, again, have only rarely and under conditions little understood been induced to germinate. Dissmann reported that his persistent effort to find sex organs of the fungus on host tissue failed, and that no oogonia came to light in numerous culture media he tried out during nearly 2 years.

A *Pythium* whose specific identity with the one Dissmann grew in pure culture as *P. undulatum* seems beyond question, developed in 9 among 42 tubes of maize meal agar that were planted late in July, 1936, with separate pieces of discolored leaf tissue taken at random from waterlily (*Nymphaea* sp.) plants growing in a drainage ditch in a cranberry bog near East Wareham, Massachusetts. After the isolation cultures were freed of bacteria and other contaminating microorganisms the fungus displayed a robust mycelial habit recalling such coarse congeneric forms as *P. ultimum*, *P. debaryanum*, and *P. anandrum*. Growing in maize meal agar its main hyphae often attain a width of 8 μ within 100 μ or 150 μ of the tip, though if the filaments are followed backward hardly any further widening is to be noted. Its lateral branches, which usually develop in moderate quantity, are of lesser thickness, and show more or less irregular secondary ramification. Despite a certain degree of coarseness, the vegetative mycelium (Fig. 26, A, B) offers the generally flexuous appearance familiar among species of *Pythium* rather than the stiffly branching aspect common in species of *Phytophthora*.

In July and August, 1944, occasion was taken to try out the fungus on young unblemished waterlily (*Nymphaea* sp.) leaves supplied from an artificial pond in Arlington, Va. Soon after their removal from the pond, the leaves were placed in large glass damp-chambers, planted with slabs excised from a maize meal-agar plate culture of the phycomycete, and stored at a temperature of 18° C. In 2 or 3 days the leaf areas under the slabs took on a dark brown discoloration. This discoloration continued to spread steadily, with the result that in 10 days it had come to extend over irregularly circular patches 40 to 60 mm. in width, though the leaves that had not been planted with the fungus still retained then their fresh green color throughout. When pieces of discolored tissue near the periphery of the brown waterlogged patches were removed to a shallow layer of distilled water in a Petri dish, and then stored at a temperature near 18° C., extramatrical hyphae 3 to 7 μ wide grew out into the liquid to produce terminally a moderate number of prolate ellipsoidal sporangia mostly 30 to 90 μ long and 20 to 40 μ wide. These sporangia on attaining definitive size were often found provided with an apical papilla, which sometimes, after renewal of the water, would form a virtually sessile cap of dehiscence. More often,

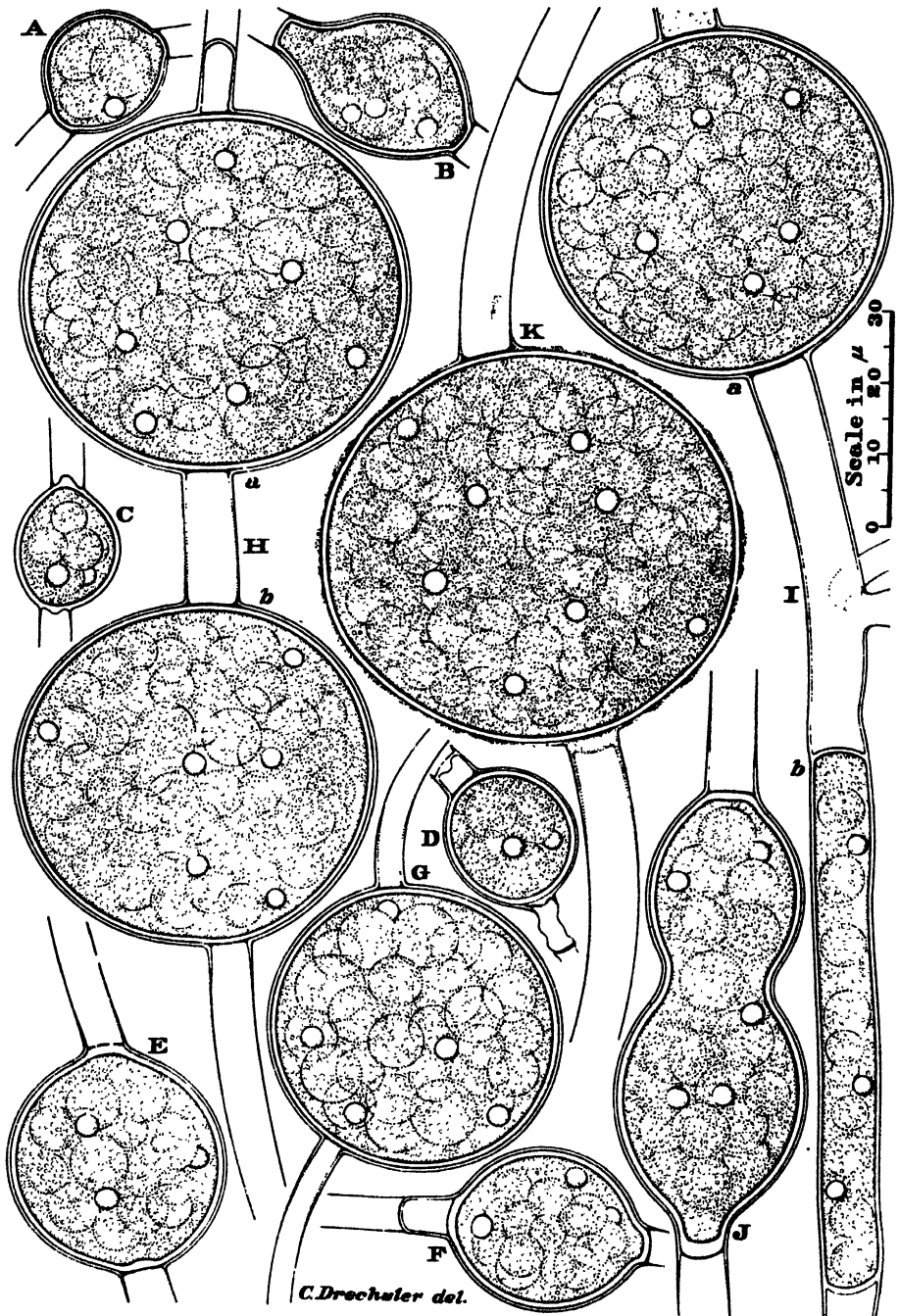


FIG. 27. Resting spores of *Pythium undulatum* Petersen *sensu* Dissmann as found in a 25-day-old maize-meal-agar plate culture; drawn with the aid of a camera lucida; $\times 1000$. A-F. Small smooth-walled specimens. G: H, a, b. Smooth-walled specimens of moderate size. I. Portion of hypha bearing a fairly well-developed smooth-walled resting spore, a, and a cylindrical resting spore, b. J. Smooth-walled resting spore of aberrant hour-glass shape. K. Rather large rough-walled resting spore.

however, the papilla grew out into a short evacuation tube before the hyaline cap was formed; so that after the undifferentiated protoplasmic contents had migrated into the inflated vesicle, and had been converted into zoospores, the empty sporangial envelope was usually found bearing distally a recognizable tubular prolongation much like the similar envelopes drawn by Dissmann from material referable to his pure culture. That the extramatrical hyphae and the sporangia really derived from the material planted on the leaves, rather than from some adventitious parasite, could hardly be doubted in view of their close resemblance to the extramatrical hyphae and sporangia produced in moderate quantity following irrigation of slabs excised from young maize-meal-agar plate cultures permeated exclusively with vigorous mycelium of the Massachusetts fungus.

More distinctive than the sporangia formed in irrigated preparations are the large globose reproductive structures or resting spores that first become noticeable in maize-meal-agar cultures about 3 or 4 or 5 days after inoculation. These structures continue development for about 20 days to present eventually a display scarcely less impressive with respect to the number of individual units than with respect to their collective bulk. In maize-meal agar of moderate nutrient content, such as I have employed, they have usually ranged in diameter from 15 to 75 μ . Occasional departures from their usual subspherical shape (Fig. 27, A-G; H, a, b; I, a; Fig. 28, A, B) are recognizable in cylindrical (Fig. 27, I, b) and in transversely constricted (Fig. 27, J) specimens. Their identity with the chlamydospores described by Dissmann becomes clearly manifest at maturity, when they are found crowded internally with an abundance of globules varying commonly from 4 to 10 μ in diameter. The smaller resting spores often contain only 4 or 5 of these globules (Fig. 27, A-D; Fig. 28, A, B), but the largest specimens (Fig. 27, I, a; K) probably contain more than 200. Their size and their distribution in a matrix of granular protoplasm provide a striking parallelism with the plural reserve globules found in the oospores of *Pythium helioides* and its allies. This parallelism gains in suggestiveness from the presence of orbicular bodies, mostly 2.5 to 3 μ wide, that are scattered presumably throughout the protoplast, even if, as a rule, they are discernible only in the upper aspect of the massive spore. For the most part these bodies appear less brilliant than the refringent bodies present singly in oospores of unitary organization, and, perhaps, somewhat less brilliant even than the plural refringent bodies in oospores of multiplicate organization; though their lack of luster could well be attributable to the feebler illumination associated with the unusual thickness of the spore.

The mature resting spores of the Massachusetts fungus, much like the chlamydospores described by Dissmann, are surrounded individually by a wall composed of 2 layers. While in some of the smallest specimens (Fig. 27, C) the two layers can be made out only with some difficulty, in specimens of moderate size they are readily seen to be distinct from one another. The outer layer is colorless and continuous with the membrane of the parent

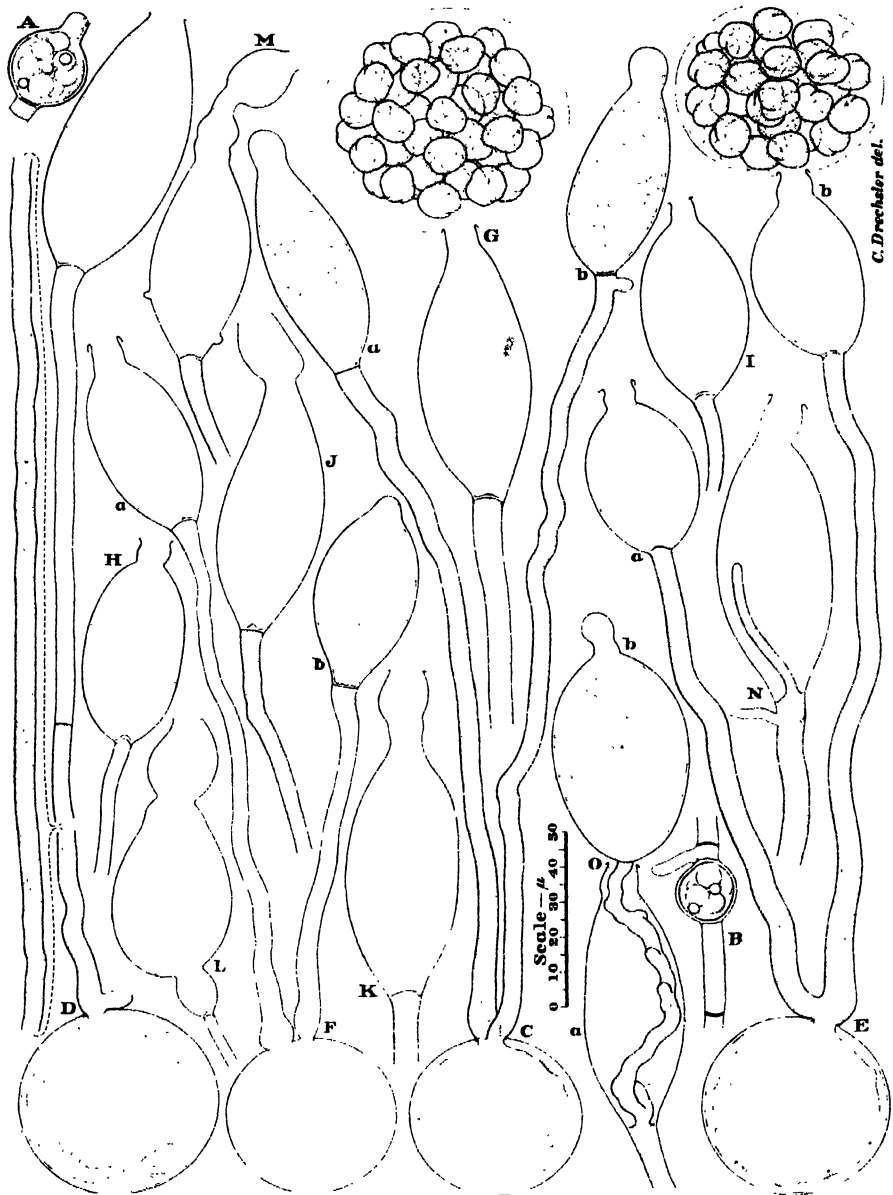


FIG. 28. *Pythium undulatum* Petersen *sensu* Dissmann; drawn with the aid of a camera lucida; $\times 500$ throughout. A, B. Very small resting spores, showing the internal organization of maturity. C-F. Resting spores from a 6-months-old maize-meal-agar plate culture, which on being transferred to water germinated by producing zoosporangia on germ hyphae: the 2 sporangia, a and b, produced by C are each extending an evacuation tube preparatory to discharge, and the resting spore retains enough protoplasmic material for the development of 1 or 2 additional sporangia; D also retains much granular material after producing a large sporangium that has given rise to zoospores; E apparently retains enough protoplasm for development of 1 or 2 more sporangia, after having produced the 2 sporangia, a and b, of which the former is represented only by its empty envelope, while the latter supports a vesicle with zoospores about ready to escape; F has contributed all its contents in producing 2 sporangia, a and b, of which the former has given rise to zoospores while the latter is still filled with granular contents. G. Large sporangium borne

hypha: the inner one, secreted by the massive protoplast, offers noticeable contrast in its yellowish coloration. In spores measuring 30 to 50 μ in diameter (Fig. 27, E; G; H, a, b; I, a) the wall commonly appears to have a total thickness of about 1.3 μ ; the outer layer usually contributing about 0.5 μ and the inner layer about 0.8 μ to the composite measurement. Many of the larger spores, including mainly specimens more than 45 μ in diameter, show a markedly irregular outer contour, and present an appearance as if they were covered with an uneven darkish incrustation (Fig. 27, K). In comparison with smooth-walled resting spores, those with a rough wall look more or less misshapen, as their subspherical form is usually found modified perceptibly by a number of broadly curved bulges. As these bulges sometimes occur in regions where the external incrustation is either very thin or wholly absent, the impression is gained that in the later stages of spore enlargement the outer membranous layer yields locally here and there or is ruptured outright in several places, permitting the somewhat elastic inner layer to push outward in the weakened regions.

Attempts to germinate newly mature resting spores of the Massachusetts fungus have always been wholly unsuccessful. When spores from a 65-day-old maize-meal-agar plate culture were transferred to a shallow layer of water in a Petri dish and stored at 18° C. a substantial proportion of them—most often between 10 and 25 per cent—germinated in the course of 7 days. On repeating the trials 100 days after the plate cultures had been planted, fully half of the spores germinated within 2 days. Virtually all spores transferred to water from cultures 180 days old germinated within 24 hours, and in strongly predominant measure germinated by the development of zoosporangia. When the resting spores contained in 0.1 to 0.2 cc. of agar from a 6-months-old culture were distributed over the floor of a Petri dish and sparingly watered, a much livelier display of motile zoospores often resulted than was obtained by irrigating ten times as much maize-meal agar or waterlily-leaf tissue permeated with young mycelium.

Preparatory to germination the resting spore takes on a somewhat opaque appearance as the reserve globules lose their clear boundaries, and together with the refringent bodies become gradually obliterated in the augmented volume of densely granular protoplasm. The inner layer of the wall dissolves away in a circular region, allowing the protoplast to protrude against the outer layer. After the outer layer has also given way the protrusion emerges to elongate externally as a germ hypha (Fig. 29, A). Apparently this germ hypha never functions directly as an evacuation tube, but like the hypha extended from the germinating oospore of *Pythium ostracodes* (25, p. 276–286) commonly forms a terminal sporangium (Fig. 29, B) from protoplasmic materials made available through increasing vacuolization

on a germ hypha and supporting a vesicle that contains approximately 45 zoospores nearly ready for escape. H–M. Empty sporangial envelopes produced in germination of resting spores. N. Empty sporangial envelope whose supporting hypha, produced in the germination of a resting spore, shows both uniaxial elongation and subsporangial branching. O. Development of 2 successive sporangia, a and b, through uniaxial elongation of the supporting hypha, which was produced in the germination of a resting spore.

within the parent spore (Fig. 28, C-E; Fig. 29, B). Where a substantial quantity of protoplasm remains, as is usually the case with large resting spores, the hypha may continue growth by putting forth a branch immediately below (Fig. 28, C, b; Fig. 29, C; E, a) or some little distance below (Fig. 29, D, a) the base of the first sporangium; the branch subsequently giving rise at its tip to a second sporangium (Fig. 29, D, b; E, b). Often 2 germ hyphae, each bearing a terminal sporangium, may be extended from well-separated positions on the resting spore (Fig. 29, F, a, b), or from positions rather close together (Fig. 28, F, a, b). Frequently, again, 2 sporangium-bearing hyphae may arise through basal branching of a single germ tube (Fig. 28, C, E; Fig. 29, G, a, b; H, a, b). Under environal conditions that favor immediate development of zoospores (Fig. 28, E, b; G) and thus permit prompt evacuation of sporangia (Fig. 28, H-M) while germination is still proceeding, the supporting filament in many instances elongates straightforwardly (Fig. 28, N) to produce a second sporangium within or beyond (Fig. 28, O, b; Fig. 29, H, c) the empty envelope of the first (Fig. 28, O, a; Fig. 29, H, a). Since in judiciously watered preparations many of the larger resting spores afford uniaxial production of 2 successive sporangia, proliferous development takes place in connection with germination on about the same modest scale as in the asexual reproduction obtained by irrigating young mycelium. Obviously no proliferous development is possible where relatively small resting spores—specimens less than 30 or 35 μ in diameter—are concerned, as these usually give rise only to a single sporangium (Fig. 29, I-L); the supporting hypha in such instances sometimes measuring less than 50 μ in length, and occasionally even less than 10 μ (Fig. 29, J). The sporangium-bearing hyphae extended from the more robust spores commonly measure 3 to 8 μ in width and 100 to 500 μ in length (Fig. 28, C-F; Fig. 29, G, H), though a considerable proportion of them may measure 0.5 to 1 mm. in length (Fig. 29, E, F) and some as much as 2.5 mm. or 3 mm.

The sporangia resulting from germinative development closely resemble those of mycelial origin in all particulars including size, since resting spores less than 30 μ in diameter are ordinarily too few to contribute any large proportion of noticeably undersized progeny. While awaiting conditions favorable for zoospore formation they are nearly always found provided with an apical papilla (Fig. 28, F, b; Fig. 29, B; C; D, a, b; E, a, b; F, a, b; G, a, b; H, b; I, J); yet now and then (Fig. 29, H, c; K), especially in the intercalary specimens (Fig. 29, L) to be seen occasionally, no distal modification is evident. The papilla sometimes is converted directly into a sessile cap of dehiscence, so that the empty sporangial envelope, after evacuation of the protoplasmic contents, will terminate abruptly in an aperture commonly 8 to 10 μ wide (Fig. 28, D), without displaying any sign of a tubular prolongation. More often, however, the papilla becomes extended into a rather short evacuation tube (Fig. 28, C, a, b; O, b) which eventually leaves its empty membrane superadded to the empty sporangial envelope (Fig.

28, E, a, b; F, a; G-N; O, a; Fig. 29, H, a). Usually the empty tube either terminates abruptly with a plain rim (Fig. 28, H, J, L, M; Fig. 29, H, a) or is minutely lipped at the orifice (Fig. 28, E, b; G; I; K; O, a), but in scattered examples it is found reflexed (Fig. 28, E, a; F, a; N) in a manner reminiscent of *Pythium vexans*. The vesicular membrane is always clearly visible. On disintegrating it releases commonly from 25 to 50 broadly reniform, laterally biciliate zoospores, which after swimming about for some time come to rest and round up into spherical cysts 9.5 to 13.5 μ in diameter (Fig. 29, M, a-z). The cysts occasionally give rise to secondary swarmers through repetitional development entailing the production of an evacuation tube usually 2 to 10 μ long and 2.5 to 4 μ wide (Fig. 29, N, a-f). More often, of course, they germinate vegetatively by putting forth 1 or 2 germ hyphae 2 to 3 μ wide (Fig. 29, O-Z).

Germination is accompanied by a marked change in the appearance of the wall surrounding the resting spore. The inner layer of the wall, which in spores of moderate size seemed earlier to measure about 0.8 μ in thickness, will usually show a thickness of 2 or 3 μ after a substantial portion of the protoplasmic contents has been contributed toward the development of germ hyphae and zoosporangia (Fig. 28, C-E). Later when the spherical chamber of the spore has been completely emptied of granular materials, the generally increased thickness of the inner layer is revealed as being varied locally by the presence of scattered pits which here and there seem to extend clear through to the outer layer. In some preparations the substance of the inner layer offers a nearly homogeneous or cartilaginous appearance (Fig. 28, F) while in others it exhibits numerous radial striations (Fig. 29, D-II) suggestive of the striations familiarly observed during germination in oospores of many congeneric species. Thus the empty two-layered envelope shows rather good correspondence with the two membranous envelopes, considered jointly, that are left from the germination of oospores in allied species; the correspondence being perhaps most obvious if comparison is made with such forms as *Pythium salpingophorum* where usually the oogonial membrane and the oospore wall are for the most part intimately fused.

The similarities shown by its membranous vestments after germination, taken together with similarities in structure of its protoplast during the long period of dormancy, provide persuasive grounds for interpreting the resting spore as a parthenospore homologous more particularly with the oospores of multiplicate internal organization that are found in *Pythium helicoides* and related species. One might be inclined to dismiss the morphological parallelism as being perhaps of fortuitous character if it were not so strongly corroborated by the physiological similarity manifest in the prolonged dormancy of the reproductive bodies under discussion; such dormancy being familiar among oospores and parthenospores, but wholly unknown among the subspherical conidia and chlamydospores formed by numerous species of *Pythium*, including, for example, *P. debaryanum* and *P. ultimum*. Kinship in the *helicoides* series would seem indicated further in the germinative

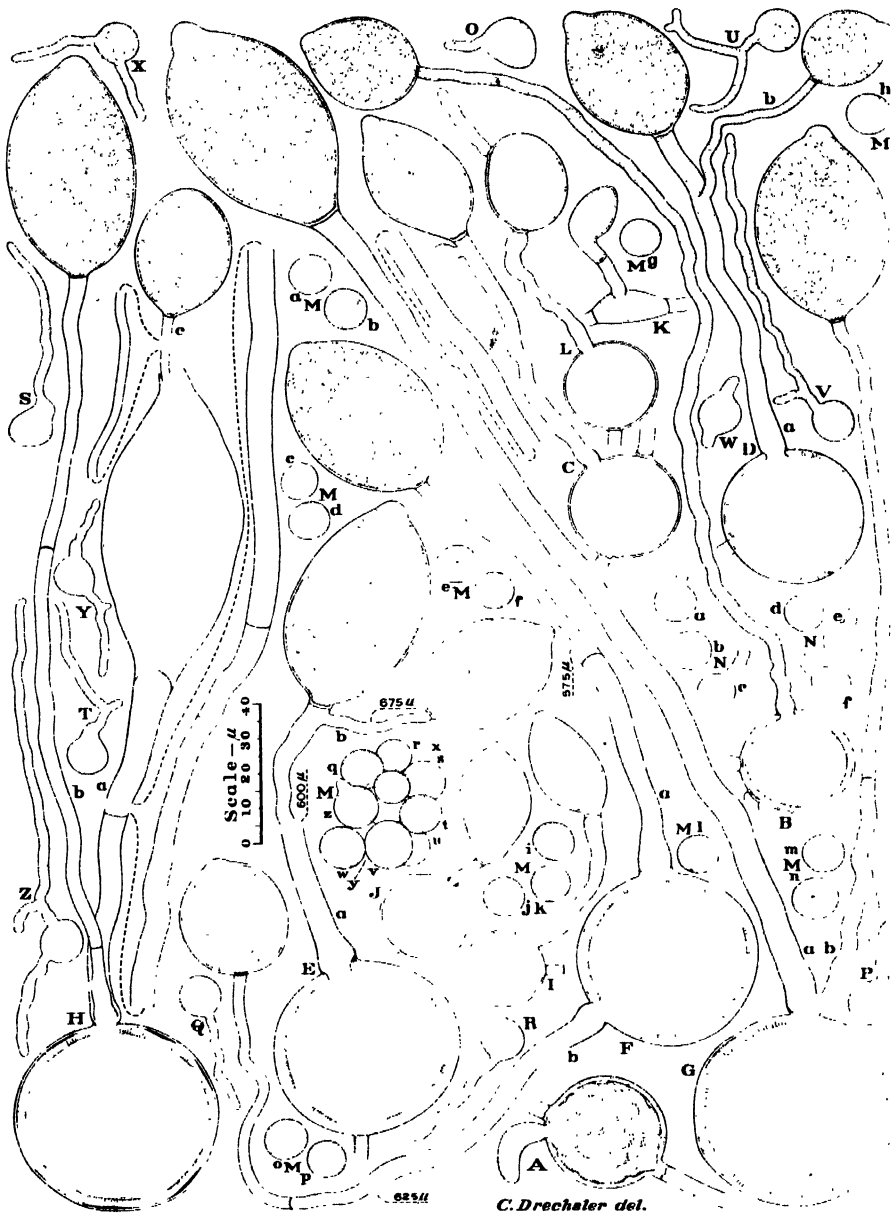


FIG. 29. Germination of resting spores of *Pythium undulatum* Petersen *sensu* Dissmann from a maize meal agar plate culture 6 months old; drawn with the aid of a camera lucida; $\times 500$ throughout. A. Small resting spore from which a germ hypha is being extended. B. Rather small resting spore that has produced a small sporangium on a germ hypha, and still retains a substantial quantity of protoplasm. C. Rather small resting spore that has produced a sporangium at the end of a germ hypha; the germ hypha thereupon branching out below the delimiting septum although containing only a small quantity of residual protoplasm. D. Resting spore whose germ hypha, a, has formed a terminal sporangium and given off a branch, b, which also supports a sporangium. E. Resting spore whose germ hypha has produced a terminal sporangium, and then put forth distally a branch, b, to bear a second sporangium; from lack of space intercalary portions of hypha measuring $600\ \mu$ and $675\ \mu$ in length, respectively, are omitted at places indicated.

behavior of the fungus; for, as has been noted, the resting spores, much like oospores of my *P. ostracodes* give rise to zoospores exclusively through the production of structurally distinct zoosporangia, never by the more direct course of development wherein the protoplasmic contents are conveyed to a vesicle by way of an evacuation tube originally extended as a germ hypha—a course of development frequent in the unitary oospores of *P. anandrum*. The zoosporangia, whether borne on germ hyphae or on mycelial hyphae, conform satisfactorily in their typically terminal position, prolate ellipsoidal shape, distal papillate modification, and occasionally successive uniaxial development, with the type of sporangium prevalent in the *helicoides* series; though this type of sporangium, it must be admitted, is characteristic also of *P. anandrum*, and besides has been recognized for many decades as distinctive of *P. proliferum* de Bary (3. p. 18, 19; 4. p. 558–562), a species which from its smooth oogonia and from the unitary internal organization ascribed to its mature oospores (3. p. 61, lines 21–25) would not seem intimately related either to *P. helicoides* or to *P. anandrum*.

Indeed, the type of sporangium here concerned—the occasionally proliferous, obliquely oriented, bursiform sporangium of my *Pythium marisipium* (23, p. 492–506) and the very meagerly proliferous, frequently sub-terminal sporangium of *P. salpingophorum*, obviously differ from it in substantial measure—occurs too widely even among terrestrial members of the genus to provide alone a really trustworthy indication of either the identity or the intimate kinship of a species. The extraordinarily large sporangial measurements given by Petersen, which might be adequate for determining the application of his binomial if they were found usual for some fungus properly referable to the genus, have assuredly not been found usual in the Massachusetts waterlily fungus, whether it was grown on artificial media or on its natural substratum, though its sporangia have regularly been of generous dimensions. However, my fungus agrees well with the one that Dissmann isolated and referred to Petersen's species; the agreement being satisfactory with respect both to the zoosporangia and to the very distinctive resting spores. Dissmann's report of zoospore formation by direct discharge of contents from very young chlamydospores is not incon-

F. Resting spore that likewise became completely evacuated in giving rise to 2 sporangia, which here, however, are borne on 2 separate germ hyphae, a and b; from lack of space portions of hypha measuring 575 μ and 625 μ in length, respectively, are omitted at places indicated. G. Resting spore that has become evacuated in producing 2 sporangia on 2 separate germ hyphae, a and b, arising from a single trunk by basal branching; from lack of space, germ hypha a is shown in parts whose proper connection is indicated by broken lines. H. Large resting spore that has become evacuated in producing 3 sporangia of which 2 were formed terminally on separate germ hyphae, a and b, arising through basal branching from a single trunk, whereas the other was formed terminally on the uniaxial prolongation c of the germ hypha a; from lack of space a and c are shown in portions whose proper continuity is indicated by broken lines. I–K. Small resting spores, each of which became evacuated in producing a sporangium on a short germ hypha. L. Small resting spore that has put forth a germ hypha with an intercalary sporangium. M. Encysted zoospores, a–z, derived through germination of resting spores, and showing variations in size and shape. N. Empty cyst envelopes, a–f, each with an evacuation tube that served in the emergence of a secondary motile zoospore. O–V. Encysted zoospores, each germinating with 1 germ tube. W–Z. Encysted zoospores, each germinating with 2 germ tubes.

sistent with my statement of germinative behavior in properly after-ripened resting spores; for, as Dissmann pointed out, the juvenile reproductive bodies he found active resembled greatly the zoosporangia formed in water, not having yet undergone, either in their protoplasm or in their envelopes, any modification tending toward the mature condition. Since he makes no mention of using material several months old in his germination trials, there is good reason to suspect that his failure with mature chlamydospores was attributable to inadequate aging.

Although the soil fungus discussed under Petersen's binomial by Matthews produced "thick-walled chlamydospores" which she held similar to Dissmann's, their small size—a range in diameter from 14 to 24 μ being given for them—would seem to make identity with my waterlily parasite quite improbable. Greater likelihood of such identity is offered by the aquatic fungus that Sparrow discussed as *Pythium undulatum*, since it gave rise on maize meal agar to "dark brown, rough-walled chlamydospores" 10 to 50 μ in diameter; though serious misgivings are aroused here by the small dimensions of the sporangia. The possibility is not to be ignored that reproductive bodies frequently rough-walled like those described by Dissmann may be formed by several members of the genus, and more particularly, perhaps, by aquatic members intimately akin to the waterlily parasite.

When the waterlily parasite is grown on maize meal-agar plate cultures in opposition to *Plectospora myriandra*, its mycelial advance is halted along the zone of encounter as its individual hyphae (Fig. 22, B, a) become enveloped by short branches extended from filaments of the saprolegniaceous form (Fig. 22, B, b, c); envelopment in all instances being followed by darkish degeneration of the protoplasm within the *Pythium* hyphae. Similar injury is sustained by the fungus when it is grown in opposition to *Pythium oligandrum*. Its hyphae (Fig. 22, C, a; D, a) on being invested with ramifying branches put forth from filaments of the spiny form (Fig. 22, C, b; D, b) soon suffer evident degeneration of their protoplasmic contents (Fig. 22, C, a), and, besides, are often invaded lengthwise by assimilative elements (Fig. 22, D). Likewise when the fungus encounters mycelium of *Pythium acanthicum* its hyphae (Fig. 22, E, a) at the forefront of advance are halted and promptly enveloped by irregular ramifications of the delicate echinulate species (Fig. 22, E, b). Often small diverticulations intruded into a newly enveloped *undulatum* hypha (Fig. 22, F, a) from an *acanthicum* branch (Fig. 22, F, b) are found surrounded by a rather thick deposit of yellow material that gives the appearance of having been secreted as a barrier against invasion. Although invasion is frequently delayed for some time, many *undulatum* hyphae (Fig. 22, G, a; H) ultimately come to be permeated by *acanthicum* filaments (Fig. 22, G, b; H). Again, when the waterlily parasite encounters a mycelium of *Pythium periplocum* its hyphae (Fig. 22, I, a) are rather extensively though not very elaborately enveloped by ramifications from filaments of the echinulate species (Fig. 22, I, b); whereupon they soon degenerate internally, and often, in addition, are invaded longitudinally by assimilative branches.

SUMMARY

Pythium oligandrum has been found frequently in damped-off seedlings as well as in decaying stems and roots of older phanerogamic plants originating from widely separated localities in the eastern United States. Its usual occurrence in association with congeneric species familiar as agents causing damping-off and root rot, together with its ready parasitism on these species, suggests that it probably operates more commonly as a secondary than as a primary invader. Once, however, it was found, unaccompanied by any other likely pathogen, in a cucumber fruit affected with watery decay in the field; and on inoculation by incision was found capable of causing decay both in nearly full-grown cucumber fruits and in watermelon fruits. Its zoosporangia resemble those of *P. acanthicum*, but appear somewhat more often to become relatively large in volume, and to include plural globose parts. The oogonium, typically subspherical and spiny, is usually delimited proximally by a massive plug and distally by a cross-wall; it often includes a cylindrical prolongation at one or at both ends, and occasionally may be wholly cylindrical. Parthenogenetic development is generally very common; its frequency varies between different strains, and, besides, is influenced by environmental conditions. Where a male complement of 1 or 2 antheridia is present, it is usually supplied from a single branch. In most instances the mycelial connection between the male and female organs is too remote to be traced. Where such connection can be traced, it often has a total length of 250 to 600 μ , occasionally a length of only 125 μ . The type of antheridium consisting of a hyphal segment adjacent to the oogonium—the type that presumably prevails in *P. artotrogus* to the exclusion of other types—has not been recognized in any material held referable to *P. oligandrum*. The oospore when mature, shows very distinctive internal organization, as it contains usually 4 to 15 refringent bodies imbedded in the granular parietal layer surrounding the single reserve globule. After a resting period of 6 months it germinates readily on shallow irrigation, often giving rise to zoospores by discharging its undifferentiated contents directly into a vesicle through an evacuation tube 10 to 50 μ long.

Oospores of *Pythium periplocum* likewise germinate readily in pure water after a resting period of 6 months. Preliminary to germination, much as in *P. oligandrum*, an inner layer of the oospore wall amounting to about two-thirds of the thickness of the envelope, is assimilated by the protoplast. The germ hypha after attaining a length of 50 to 200 μ frequently functions as an evacuation tube in conducting the granular contents into a vesicle where they are fashioned into zoospores.

Pythium salpingophorum requires a lower temperature for zoospore production than most congeneric species. In irrigated preparations its globose sporangia are more often formed in subterminal than in terminal or intercalary positions, and are only in rather small measure given to successive development through either uniaxial elongation or subsporangial branching of the supporting hypha. The species is distinguished by pronounced distal

widening of the evacuation tube; the empty membrane of this tube becoming reflexed at the orifice somewhat in the manner of a trumpet. The rather small, smooth, subspherical oogonia frequently develop parthenogenetically, but many are supplied with 1 or 2 antheridia which may be borne on a short branch arising from the oogonial filament in close proximity to the oogonium, or, again, may be sessile either on the oogonial hypha or on a neighboring hypha. Except in the proximal and distal regions the oogonial envelope is usually adnate to the oospore wall, which at maturity encloses a protoplast of unitary organization—a single refringent body being imbedded in the granular layer surrounding the single reserve globule. After a resting period of 8 months the oospore germinates freely in pure water, often through the production of swarmer. The protoplast generally assimilates a thick inner layer of the oospore wall before putting forth a germ hypha that sometimes functions directly as an evacuation tube and at other times bears terminally a sporangium similar to sporangia of mycelial origin.

In *Pythium vexans* (= *P. complentens*), after discharge of the sporangium, the empty membrane of the evacuation tube is often though not always reflexed at the open end. As the oogonium and antheridium in this species are brought together at a very early stage, they necessarily expand in intimate contact with one another. Where a hard agar culture medium offers considerable resistance to their expansion, their outward shapes are noticeably modified; the oogonium becoming flattened or broadly indented in the region of contact, the antheridium at the same time being squeezed to fit snugly until in extreme instances it appears as an irregularly lobed mass. However, in water or soft agar the subspherical shape of the oogonium undergoes little modification, while the antheridium develops rather often into a ramified body consisting of 2 to 4 elongate digitate or more broadly lobate parts that clasp the oogonium extensively. The antheridium is regularly borne terminally on a branch arising either from a neighboring hypha or from the oogonial hypha at some distance from the oogonium. Since its base is often very close to the oogonial attachment, it frequently has much the appearance of being sessile on the oogonial filament close to the oogonium. Oospores of *P. vexans* soon germinate freely in pure water, often giving rise to swarmer. The protoplast, after assimilating a thickish inner layer of the oospore wall, sometimes extends a germ hypha that functions directly as an evacuation tube, and at other times produces a structurally distinct zoosporangium which may be sessile on the oogonial envelope or may be terminal on a germ hypha of variable length.

On shallow irrigation, oospores of *Pythium anandrum* from cultures 3 months old germinate readily, usually giving rise to swarm spores. After absorbing a thick inner layer of the oospore wall the protoplast often puts forth a stout germ hypha that subsequently functions directly as an evacuation tube. In other instances a zoosporangium structurally distinct from the oospore is produced terminally on a germ hypha up to 200 μ in length. Such a sporangium may also be formed sessile on the oogonial envelope, or

may even be deeply inserted into the chamber of the oospore. Repetitional development of zoospores is accomplished in *P. anandrum* usually, as in *P. oligandrum*, *P. vexans*, and *P. undulatum*, by direct production of an evacuation tube, but also takes place occasionally through production of a miniature sporangium.

A fungus isolated from waterlily leaves in Massachusetts is referred to *Pythium undulatum* Petersen *sensu* Dissmann by reason of its prolate ellipsoidal, distally papillate sporangia and its large rough-walled resting spores. In their mature condition the resting spores show an internal organization similar to that in oospores of *P. helicoides*, and are surrounded individually by a wall consisting of a thin (0.5 μ) colorless layer and a somewhat thicker (0.8 μ) yellowish inner layer. Resting spores from cultures 6 months old germinate readily on shallow irrigation by producing 1 to 4 sporangia on germ hyphae 10 μ to 3 mm. long. After germination the inner layer of the spore wall appears much thicker (2 to 3 μ) than before. It seems probable that the resting spore represents a parthenospore homologous with the oospore of *P. helicoides*.

When *Pythium salpingophorum*, *P. vexans*, *P. undulatum*, and *P. anandrum* are grown on an agar substratum in opposition to *P. oligandrum* or *P. periplocum* or *P. acanthicum*, their mycelial advance is halted as their hyphae in varying measure become enveloped by branches extended from the filaments of the opponent fungus; the enveloped hyphae usually soon showing degeneration of their contents, and often in addition undergoing invasion by assimilative elements. Similar injury is sustained by them when they are grown in opposition to *Aphanomyces cladogamus* or *Plectospora myriandra*. *P. vexans* appears occasionally to retaliate upon *P. periplocum* by applying appressoria to hyphae of the echinulate form.

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A NEW BACTERIAL LEAF SPOT OF GREENHOUSE-GROWN GARDENIAS

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In central California heavy losses are being sustained in some gardenia plantings on account of a bacterial leaf-spot disease which is similar in symptoms to that described by Burkholder and Pirone.¹ However, careful study of the causal organism of the California disease proved it to be different from that described in the eastern United States.

The disease starts on tender leaves as minute dots which gradually enlarge, showing at first a pale yellow center, which later becomes reddish-brown, surrounded by a yellowish halo. The margins of the lesion are somewhat thickened and have a greasy appearance. Several spots may coalesce to form a larger one. Infections when very abundant (Fig. 1, A and B) may cause premature abscission, an unfavorable condition from the production standpoint. Flower buds and sepals are sometimes affected (Fig. 1, C and D), but this is relatively uncommon.

The disease may be spread by cuttings taken from infected plants, and especially by syringing plants to reduce the red spider mite population. When gardenias are forced for flower production, the high humidity and air temperature usually maintained provide excellent conditions for the spread of the organism and subsequent development of epidemics.

Microscopic examination of small pieces of diseased leaf tissues reveals an abundance of bacteria oozing from the cut edges. If the bacteria diffuse into sterilized water and subsequently dilution plates are made, the organism can easily be isolated in pure culture.

The pathogenicity of the bacterium (six isolates representing six different tests) isolated from the gardenia leaf spots was tested by suspending the isolated bacteria in sterile distilled water and atomizing it on small potted gardenia plants. Infection occurred and the bacterium isolated from the artificially produced lesions was identical with the one used for the inoculations. Prior to inoculations the plants were held in a large moist chamber for 24 hours and were kept there for the duration of the test to conform to commercial conditions of growing this crop as close as possible. First symptoms of the disease, in a form of very small pale yellow spots, appeared from 7 to 9 days after the spraying, the temperature of the greenhouse varying from 80° to 90° F. during the day, with night temperature about 65° F. In two weeks the spots developed to the size of 1 mm. Keeping the atmosphere and the foliage dry checks the disease promptly and new leaves are free of the disease. Resumption of syringing coupled with high temperature is soon followed by infection on new foliage.

¹ Burkholder, W. H., and P. P. Pirone. Bacterial leaf spot of gardenia. *Phytopath.* 31: 192-194. 1941.

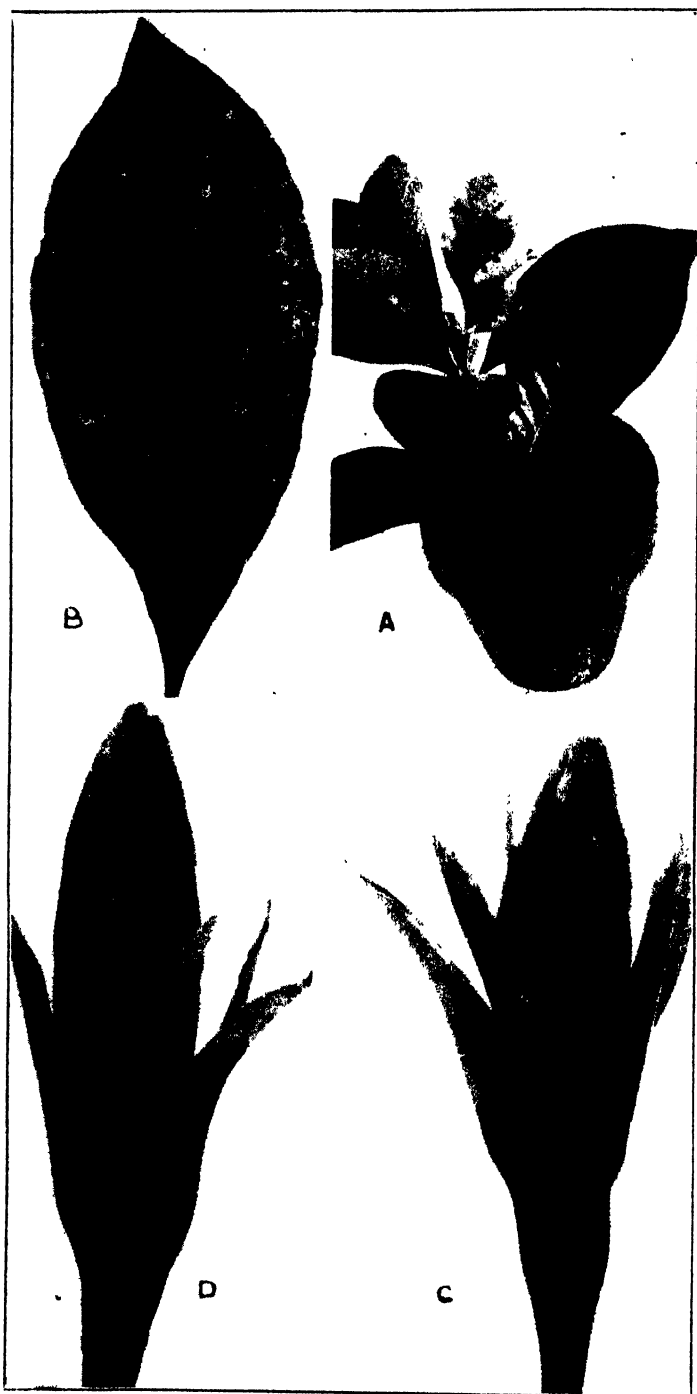


FIG. 1. Bacterial leaf spot of gardenia caused by *Phytophthora maculifolium-gardeniac*. A and B. Natural infection on the leaves; C. Petal infection; D. Sepal infection.

The bacterium causing this disease is a short rod measuring $1.6-2 \mu \times 0.3-0.5 \mu$, motile by means of one or two flagellae attached to one pole. It is stained by most of the anilin dyes and is Gram-negative. In some preparations, the capsule can be demonstrated by the India ink method.

In beef-extract-peptone broth the turbidity is prompt in 24 hours, becoming dense in time with a narrow yellow ring at the top and a viscous yellow sediment at the bottom. On beef-extract-peptone agar, the growth is rapid, slightly raised, yellow (Pyrite yellow according to Ridgway standards), butyrous in young cultures and difficult to pick up in older tubes. On potato-dextrose-peptone agar slants the growth is fast, yellow (sulphine yellow), smooth, and falls to the bottom of the tube in time. Growth is good in 48 hours in Cohn's synthetic medium, in Ushinsky's medium, and in the synthetic carbohydrate medium of the Society of American Bacteriologists;² but growth is scanty in Fermi's solution. Starch plate is well digested after 48 hours and completely digested after 6 days. Indol and hydrogen sulphide are not produced. Ammonia is formed in peptone-glucose-dipotassium phosphate media devised by Hansen³ and demonstrated by his method; nitrates are not reduced. The skimmed milk becomes translucent with a white precipitate at the bottom and a large yellow ring at the top. In litmus milk, a white curd is formed at the bottom and the supernatant liquid is a dirty wine color. Gelatin is liquefied slowly.

Growth in synthetic media containing the following carbohydrates produced acid and no gas: arabinose, dextrose, fructose, galactose, lactose, maltose, mannite, raffinose, sucrose, and xylose. An inverted Dunham's fermentation tube was added to each tube to demonstrate presence or absence of gas. Glycerine was not utilized by the organism.

The organism grew from 10^2 to 37°C . No growth of the organism was observed even after 30 days at the temperatures from 1° to 7°C . At 10°C the growth was slow. The optimum temperature for growth lies between 22° and 28°C . The thermal death point is 50°C .

Since no known yellow pathogens have similar characters, the organism is deemed to be new to science and the name *Phytomonas maculifolium-gardeniae* n. sp. is proposed. It differs markedly from *Ph. gardeniae* which produces white colonies becoming dirty in appearance with dark-brown discoloration of the medium.

To control the disease numerous experiments were undertaken. Spraying with standard Bordeaux mixture gave no control. Various quaternary ammonia compounds were ineffective. However, an aqueous copper sulphate solution, 1 to 2000, with the addition of a spreader (0.1 per cent Triton B-1956 phthalic glyceryl alkylid resin), reduced the number of lesions. This spray should be applied at frequent intervals to prevent any build-up of the disease on old leaves. Syringing of the plants for the control of

² Society of American Bacteriologists, Manual of methods for pure culture study of bacteria. P. 14, Leaflet 2. Preparation of media. 9th ed. 1944.

³ Hansen, P. A. The detection of ammonia production by bacteria in agar slants. Jour. Bact. 19: 223-229. 1930.

red spiders definitely aggravates the disease. Therefore attempts should be made to control red spiders by other means. Lately, the use of Azofume-70 (technical grade of azobenzene) as a fumigant for red spiders was found successful by some gardenia growers and resulted in a considerable reduction of the disease.

SUMMARY

1. Leaf spot on gardenia in California is caused by a yellow bacterium, for which the name *Phytomonas maculifolium-gardeniae* is proposed.

2. The disease increases to serious proportions under conditions of high humidity and high air temperatures.

3. In controlling the disease it is important to avoid syringing the plants with water. They should be sprayed at frequent intervals with aqueous copper sulphate solution, 1 to 2000, plus a spreader.

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THE SOIL ROT OF SWEET POTATOES AND ITS CONTROL WITH SULPHUR

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The soil rot of sweet potatoes caused by *Actinomyces ipomoea* Person and Martin is at present widespread in Louisiana as well as in other parts of the United States. In Louisiana, it was first observed in a serious form in 1934 in small, isolated areas near Sunset in St. Landry Parish, the center of the sweet-potato-growing area. In later years, the areas gradually enlarged and the disease also appeared in other parts of the State.

When weather conditions are favorable and the disease is severe, it causes almost a total loss. Many roots are destroyed and the young plants either die or their growth is so checked that vines are not produced. When conditions are less favorable for the disease, a satisfactory yield may be obtained, but the quality is poor because many potatoes are misshapen or covered with unsightly lesions.

A very severe outbreak in 1937 was largely responsible for the investigations here reported. Studies between 1934 and 1937 had indicated that the disease was not serious in a soil with the pH below 5.2. Preliminary experiments also had indicated that it was possible to lower the pH of the soil to 5.0 by adding sulphur, but it was not known how much sulphur would be required, or how long the low pH would be maintained in the soil after the sulphur was applied, or whether a pH this low would injure sweet potatoes or other crops that might be grown. Sulphur tests have been made for eight years, in seasons both favorable and unfavorable to the soil rot, and the results obtained have provided definite answers to these questions.

MATERIALS AND METHODS

Plots were sulphured on 20 farms in three areas in St. Landry Parish. With the exception of one farm, the soils were Lintonia silt loam, the typical first terrace soils of that area. The soil of one farm was a Miller silt loam, a reddish soil similar to the alluvial soils of the Red River. The terrace soils are found on the greater portion of the farms in the sweet-potato area in St. Landry Parish. The area with the Miller silt loam is very small. The pH of the terrace soils is ordinarily about 5.7, and of the Miller soil about 6.1.

The general procedure for applying sulphur was the same in all tests. After the field was leveled by plowing or discing, sulphur was applied with a seed drill and disced in as soon as possible. The soil was then thrown up into the 4½-foot, ridged rows, the system ordinarily used in that area. The sulphured plots were usually 7 to 10 rows wide, although in one or two tests they were only 5 rows wide. The nonsulphured check plots were 5 or

¹ The author wishes to acknowledge the assistance of the Freeport Sulphur Co. for donations of sulphur and for a grant to help finance the field work; and the Southern Acid and Sulphur Co. for donations of sulphur.

more rows wide. All plots were 200 feet, or more, long. Five types of sulphur were used:

1. Commercial flour; a sulphur of which 90 per cent passes through an 80-mesh screen.
2. Stauffer granular; a sulphur which passes through a 16-mesh screen.
3. Toro; a conditioned sulphur containing 14 per cent filler, 93 per cent of which passes through a 325-mesh screen.
4. Soil sulphur; a ground crude sulphur of 99.5 per cent purity.
5. Inoculated sulphur; a sulphur to which sulphur bacteria had been added.

Soil samples were collected periodically from each plot and pH readings were made. After a plot was sulphured, samples were collected several times in the first six months to determine the time necessary for the pH to drop to 5.0 or below. After that, soil samples were taken one or more times a year until 1945, or until the plots were out of cultivation. From 1938 through part of 1941, the Hellige colorimetric method was used in making the pH tests. After that, a Beckman potentiometer was used. Slightly lower readings were obtained with the potentiometer.

When the sweet potatoes were harvested, the three center rows in each plot were dug and the potatoes graded and weighed. The yields per acre were then calculated in 50-lb. crates of No. 1 and No. 2 sweet potatoes.

EFFECT OF SULPHUR AND ACIDS ON pH OF SOIL

To determine the amount of sulphur necessary to bring the pH of the soil to 5.0 and to maintain it at that low level, four rates of application per acre were used: 500 lb., 600 lb., 700 lb., and 800 lb. The effect of these amounts on the pH of the terrace soils for 7 years is given in table 1. As similar results were obtained with all types of sulphur, the results are grouped in the table. The soil sulphur was slightly slower in action, taking about a month longer to bring the pH down to the level produced by the others; but after that the results with all were similar. The data in the table represent averages. The 500-lb. application was sufficient to lower the pH to a satisfactory level but did not maintain it. As the pH in the plots with the 500-lb. applications tended to rise after the first year, the applications in later years were mostly 600 lb. or more. The 600-, 700-, and 800-lb. applications all held the pH to 5.0 or below for about four years, after which it tended to rise to 5.2 to 5.4. Usually it took from two to four months after the sulphur was applied for the pH to drop to 5.0.

Results obtained on the Miller silt loam were quite different from those on the terrace soils. Applications of sulphur, even up to 800 lb. per acre, barely lowered the pH to 5.0 within the first six months. This low level, however, was not maintained. The pH began to rise gradually and within a year was 5.4 or above, and it continued to rise in later years. The lowering of the pH to 5.0 by the application of sulphur did not seem practical on this soil and further applications were not made.

Concentrated sulphuric acid and phosphoric acid were also applied to the terrace soils at rates of 400 to 700 lb. per acre. These had very little effect on the pH of the soil in the months that followed.

TABLE 1.—*Effect of sulphur on soil pH on 19 farms in St. Landry Parish, Louisiana*

Sulphur applied per acre	Period after sulphuring								
	1-2 mos.	3-4 mos.	5-6 mos.	1 yr.	2 yr.	3 yr.	4 yr.	5 yr.	6 yr. 7 yr.
No. sulphur	5.7	5.8	5.8	5.9	5.7	5.6	5.7	5.7	5.7
500 lb.	5.2	4.9	4.9	5.0	5.2	5.3	5.1	5.1	5.2
No. of plots	12	14	20	20	11	5	5	5	4
600 lb.	5.1	4.9	4.7	4.9	4.9	4.9	5.0	5.2	5.4
No. of plots	89	93	95	98	98	87	83	45	6
700 lb.	5.1	4.9	4.7	4.8	4.8	5.1	5.0	5.0	4.8
No. of plots	16	18	24	24	24	15	9	3	3
800 lb.	5.1	4.9	4.6	4.8	4.8	4.8	4.9	5.2	5.2
No. of plots	102	101	108	108	108	101	94	61	14

RELATION OF RAINFALL TO SOIL ROT

Observations in Louisiana as well as elsewhere have indicated that there is a definite relation between rainfall and the severity of soil rot. The disease may be severe in seasons with periods of drought, especially if these occur within a month after planting, which is the time the roots are developing on the young plants. The disease may be entirely unnoticeable in years with sufficient rainfall well distributed throughout the growing season. In the sulphur tests here reported, the amount and the distribution of the rains were very important in evaluating the results obtained.

In Louisiana, sweet potatoes are planted at any time during the spring and early summer, but in recent years, in order to increase yields and also to have marketable potatoes during the early fall months, there has been a tendency to plant as early in the spring as possible. Because of this, most of the crop is planted in May or early June. The actual date of planting, however, depends to a great extent on the condition of the soil, as sufficient moisture must be present to enable the plants to establish their root systems.

Weather conditions in the spring in Louisiana vary considerably from year to year. On the average, there is less rain in May and early June than in later months, and often there are periods of two to three weeks or more without sufficient rainfall to meet the requirements of the young sweet-potato plants. When such conditions occur, the roots become so severely injured by soil rot that they do not recover satisfactorily, even though the rain later is ample. For planting in May, the critical rainfall period seems to be the last half of April, all of May, and the first half of June.

Results obtained in the sulphur tests can be evaluated better if they are considered with the rainfall data. As reported by the U. S. Weather Bureau, the rainfall at Grand Coteau, a station close to Sunset, during the critical spring period for the years between 1933 and 1945 is presented in table 2. The classification, as to wet, dry, or normal, is based on the opinions of field

workers. The sulphur tests were made between 1937 and 1945, but the earlier years are included in order to explain the earlier occurrences of soil rot.

TABLE 2.—*Rainfall at Grand Coteau, Louisiana, for spring planting seasons, 1931-1945*

Year	Apr. 16-30	May 1-15	May 16-31	June 1-15	How classified
1933	3.38	1.72	3.90	2.54	Normal
1934	2.12	1.02	2.19	1.49	Dry
1935	4.37	11.70	1.52 ⁵ / ₈	2.99	Wet
1936	1.37	0.61	6.97 ⁵ / ₈	0.06	Normal
1937	0.20	2.98	0.94	2.71	Dry
1938	2.66	0.84	2.31	1.29	Dry
1939	0.71	0.93	4.06	3.88	Normal
1940	8.16	0.42	0.65	5.69	Wet
1941	6.11	4.70	4.45	15.89	Wet
1942	0.00	0.88	1.77	6.81	Wet
1943	1.43	1.63	2.33	0.00	Dry
1944	2.14	2.00	3.84	0.96	Dry
1945	1.49	0.33	4.27	4.22	Normal
50-yr. average	2.18	2.75	2.75	2.53	

Between 1933 and 1945, there were several years that were abnormally dry or years in which, at some time during the spring, there were long periods with but little rain. These would include 1934, 1936, 1937, 1938, 1943, and 1944. Of the others, 1935, 1940, 1941, and 1942 were definitely wet in the periods following planting. As rains are often local, weather reports do not always give the actual condition in a particular field. Outbreaks of soil rot that have occurred in the State and have caused concern to the growers have been in the years listed as dry years. The outbreaks which occurred in 1936 and 1937 were definitely responsible for the present investigation.

EFFECT OF SULPHUR ON YIELDS

During the eight years, yields were obtained from as many of the sulphured plots as was possible. In the Sunset area, a crop rotation is followed and so, in most cases, sweet potatoes were grown on the sulphured plots only once in every two or three years. In the eight years, yield data were obtained from 60 tests. These tests were not entirely comparable, being made in different years under various weather conditions and in fields in which sulphur had been applied in a varying number of years preceding the tests. Also, in some tests, it was not possible to set out all the plants at the same time. However, it is felt that, with the exception of those obtained in 1940, the results presented are reasonably representative and show fairly accurately what may be expected from applying sulphur on fields infested with the soil-rot organism. In 1940, after a flood in August, many of the fields were under water and the plants in both sulphured and nonsulphured plots were badly injured.

The results obtained during the eight years are in table 3. These include

all tests, those made the first year after applying the sulphur as well as those made from two to eight years after applying the sulphur.

TABLE 3.—*Average yields per acre, in 50-lb. crates, on sulphured and nonsulphured plots*

Year	No. of tests	Rate of sulphur applied per acre					Moisture condition
		No sulphur	500 lb.	600 lb.	700 lb.	800 lb.	
1938	8	8.0	77.4	99.1	66.9	83.3	Dry
1939	9	112.8	138.5	133.2	124.7	111.7	Normal
1940	10	57.9		74.7	69.4	63.5	
1941	12	123.0	116.9	115.2	186.5	113.1	Wet
1942	5	156.3	139.0	166.0	137.1	156.0	Wet
1943	5	56.8	94.1	114.5	123.6	125.2	Dry
1944	6	35.4		156.7		177.9	Dry
1945	5	81.1	102.6	127.4	111.6	149.3	Normal

In evaluating the results, it should be recognized that they are not strictly comparable, and on this account variations with the different rates of application should not be considered significant. The tests were in different years, some wet and some dry; often it was not possible to plant all plots in a single test at the same time; in some instances, soil moisture was favorable at planting time and in others it was not; often the infestation with the *Actinomyce* was not equally distributed in the field and, on this account, the plants in some plots were more severely injured by the pathogen than those in others. The tests, however, were on a large scale and the results are reliable. It is believed that they give a fairly accurate idea of what was obtained and what may be expected in the future with the sulphur treatment.

It was recognized in the early years of the investigation that the increases in yield from the 500-lb. applications of sulphur were not so great as those with the applications of 600 to 800 lb., and in the later years the higher applications were used almost exclusively. However, significant increases were obtained with all of the applications. In dry years these increases were very large.

As the increases from the different treatments were not very different, there are presented in table 4 the average increases obtained from the sulphur treatments in dry and wet years and also in years considered fairly normal from the standpoint of precipitation.

TABLE 4.—*Increases in yield, measured in 50-lb. crates per acre, resulting from sulphur treatment in wet, dry, and normal years; all treatments and tests averaged*

Rainfall conditions	Average yields in crates per acre		Increases in crates per acre	Percentage increase
	No sulphur	Treated with sulphur		
Dry years	33.4	111.9	78.5	235.0
Normal years	96.9	124.8	28.1	29.0
Wet years	139.6	141.2	1.6	1.2

The results obtained in the so-called dry years, or in those years in which the soil was dry during the period when plants were producing their roots and becoming established, were very outstanding. In such years, on many of the nonsulphured plots, the loss was total or practically so. Only in those plots in which the infestation of the Actinomycete was apparently restricted, did any of the plants grow and produce potatoes. In the sulphured plots, the yields were satisfactory and as good as or better than the average of the fields in the community not affected with the soil rot. Sulphur was responsible for an average increase of 78.5 crates per acre, or 235 per cent. In some tests, increases of 120 to 160 crates were obtained. In what were considered normal years, an increase of 28.1 crates or 29 per cent was obtained. In the wet years, however, the sulphur seemed to have little or no effect.

HOW LONG IS SULPHUR EFFECTIVE

From the standpoint of the grower, it has been important to determine the effectiveness of a single application of sulphur in controlling soil rot in the succeeding years. If sulphur were only effective in holding the disease in check in the season immediately following its application, its use might not be a practical method of control, because of the expense involved. If, however, satisfactory sweet-potato crops could be grown in the treated fields for six or eight years, the expense of treatment that might be charged to each crop would be small.

The tests in the last few years of the investigation were to determine the lasting effects of the sulphur. No new applications of sulphur were made and the plots used were those which had been sulphured from 4 to 8 years earlier. The yields obtained in the individual tests in 1944 (a dry year) and 1945 (a normal year) with the number of years after the applications of sulphur are included in table 5. Only the plots which received 600 lb. of sulphur are included.

TABLE 5.—*The effectiveness of sulphur treatment in years following application. Yields of nonsulphured plots and plots treated with 600 lb. sulphur in individual tests in 1944 (dry year) and 1945 (normal year).*

1944			1945		
Crop year after treatment	No sulphur	Treated with sulphur	Crop year after treatment	No sulphur	Treated with sulphur
4	0.0	112.5	5	134.5	153.2
4	1.8	118.4	8	44.1	121.0
5	82.1	204.0	6	168.3	200.7
4	6.7	129.4	5	32.3	107.9
4	18.9	187.2	6	26.1	54.2
6	102.8	188.7			

It seems certain that on the terrace soils of St. Landry Parish in Louisiana, applications of 600 to 800 lb. of sulphur will largely eliminate losses from soil rot for five to six years and possibly longer. While it has not been

determined with certainty how long a sulphur application will be effective in controlling the soil-rot organism, it has seemed advisable to recommend a second application of sulphur at the rate of 200 to 300 lb. per acre after five to six years. Such an application seems necessary to hold the pH of the soil close to 5.0.

SUMMARY

The soil rot of sweet potatoes has caused serious losses in certain areas in Louisiana since 1934.

The causal organism, *Actinomyces ipomoea*, is not important at a pH of 5.0 or less. The terrace soils in St. Landry Parish in which the disease has been troublesome vary from pH 5.6 to 5.8.

By applying sulphur at rates of 500 to 800 lb. per acre, it has been possible to lower the pH of these soils to about 5.0 and to maintain it at that level for four to six years. The 500-lb. application was not so effective as the higher applications.

Losses from soil rot have been practically eliminated by the application of sulphur. In the better fields, increases of 120 to 160 crates per acre from the sulphur-treated areas have not been uncommon.

The disease was more severe in years when rainfall was low at the time the young plants were producing roots. In dry years in severely infested fields, all plants were killed, or failed to produce good vines. In very wet years, losses from soil rot were slight.

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THE CYTOLOGY OF *USTILAGO STRIIFORMIS* FORMA *POAE-PRATENSIS* IN ARTIFICIAL CULTURE¹

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INTRODUCTION

Stripe smut of grasses, *Ustilago striiformis* (West.) Niessl, is known to be parasitically specialized and at least five physiologic forms have been recognized (4, 5). The physiologic forms are distinguished primarily on the basis of host specificity, although marked differences in methods of spore germination, cultural behaviors, and life histories have been reported (4). Leach, Lowther, and Ryan (13) called attention to certain of these differences and have described the unique behavior of the stripe smut from bluegrass (*Poa pratensis* L.) when grown on agar. They showed that this smut readily forms chlamydospores on potato-dextrose agar and has two distinct types of vegetative growth.

The first isolations of this smut were obtained from tissue cultures made from unruptured pustules and the immediately surrounding host tissue. Two types of colony were observed in the original plates, one consisting of straight, radiating hyphae that formed a tough, leathery, mycelial mat, the other being composed of irregular, curved hyphae that, when disturbed, broke readily into numerous short fragments. The latter type of colony has a wrinkled topography and is waxy in texture. Spores were formed by both kinds of colony, but were formed sooner and more frequently by the fragmenting type. Although the origin of the first colonies isolated was not noticed, microscopic observations made of later isolations indicated that the fragmenting colonies arose chiefly from mycelial fragments from immature pustules, while the radiating colonies arose from germinating spores. Germinating spores, however, may eventually give rise to fragmenting colonies.

Because of the unusual ability of this smut to complete its life history in artificial culture and because of its peculiar types of vegetative growth, a cytological study promised to be of more than ordinary interest. It is the purpose of this paper to describe the cytology of the fungus as studied in artificial culture.

MATERIALS AND METHODS

An effort was made to stain the fungus with a nuclear stain in all stages of growth from early spore germination to the formation of mature chlamydospores. Spores were germinated in distilled water, in dilute malt extract, and on a thin film of agar spread over the surface of a slide according to the method used by Wang (15). The last method, in general, gave the most satisfactory results. The various stages of vegetative growth were obtained

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partly by allowing germinating spores to grow for various periods of time on the agar film and partly by taking material from Petri-dish cultures of different ages and spreading it over slides covered with egg-albumin fixative. The material was killed and fixed by exposure to the vapor of Flemings' weaker solution for 10 to 20 minutes. The fixed material was stained with fairly satisfactory results by using iron-alum haematoxylin and following, with minor variations, the schedules of Holton (10) and Hirschhorn (9).

RESULTS

As previously reported (13) the chlamydospores germinate by forming a germ tube that usually branches before it has become more than $100\ \mu$ long. The germ tube branches sooner and more profusely in a nutrient solution

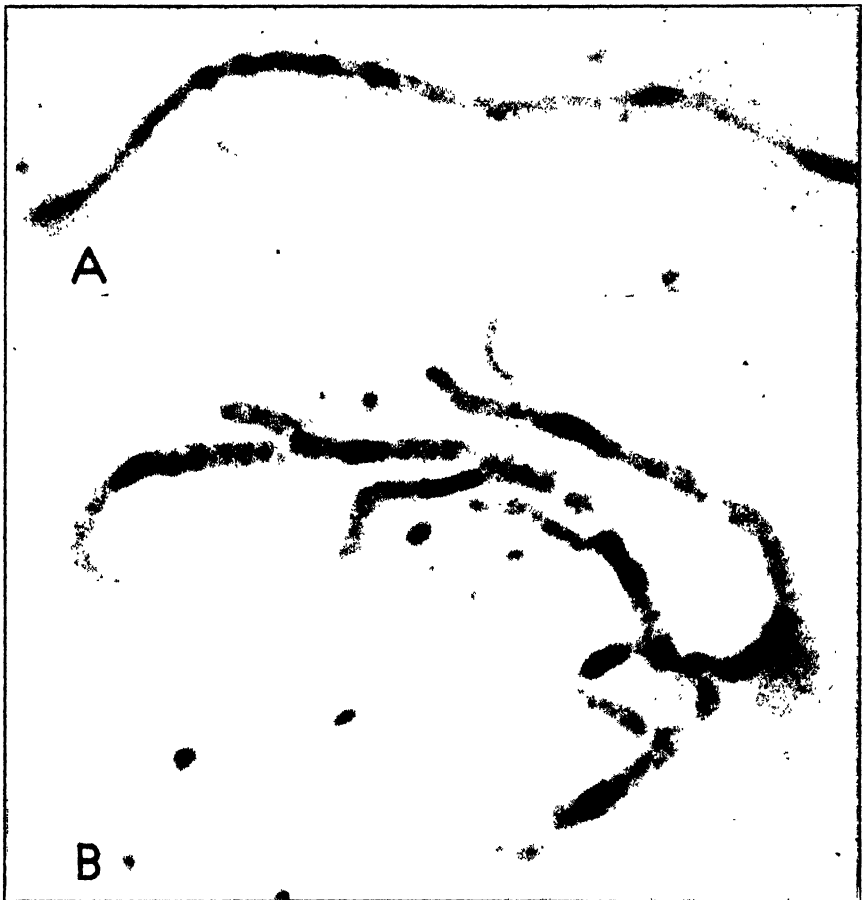


FIG. 1. A. An unbranched germ tube containing four nuclei whose size and characteristic grouping indicate that they originated through reduction division. Stained with iron alum haematoxylin. Approx. 1200 \times . B. A segment of a diploid (syngaryotic) mycelium showing the large oblong diploid nuclei and the thick, transparent septa, which, upon dissolving, cause the mycelium to break up into short fragments. Approx. 1200 \times . Note the relative size of the nuclei in A and B.

than in distilled water. Occasionally two distinct germ tubes are formed, usually arising from opposite sides of the spore. The germ tube is non-septate and produces no sporidia. Without noticeable pause, the branched germ tube, when growing on agar or in a nutrient solution, grows directly into an extensively branched mycelium. Thus, the growth of the germ tube is indeterminate, and the promycelium, characteristic of most smuts, is completely lacking.

The nuclei stained well and were readily recognized. Because of the dark color of the spore wall, the behavior of the nucleus within the spore could not be determined clearly. Apparently the spore contains one large fusion nucleus, as reported for most other smuts. This nucleus divides early in the process of germination. Usually it divides after entering the germ tube but it may divide while still within the spore. The latter is evidently the case when two separate germ tubes are formed. The first division is followed immediately by a second; hence, four nuclei in orderly arrangement are very common when the germ tubes are stained at the proper time (Figs. 1, A, and 5, A, B, C). All stages from one large fusion nucleus to the four smaller, apparently haploid nuclei are found in young germ tubes (Fig. 5, A-F). This nuclear behavior is interpreted as evidence of the occurrence of reduction division, although individual chromosomes and their behavior could not be recognized with any degree of certainty or consistency.

The nuclei in young germ tubes are nearly spherical and measure 2 to 3 μ in diameter. There is, however, considerable variation in size and shape in different preparations. Although the nuclei were well differentiated, the behavior of individual chromosomes could not be followed. In many individual nuclei chromosome-like bodies could be detected, but it was only by the exercise of much imagination that their behavior could be interpreted as meiosis. If the observed chromosome-like bodies are true chromosomes, the chromosome number in the haploid nucleus is, in all probability, two. In many cases, however, nuclei appearing to consist of two chromosomes could equally well have been interpreted as nuclei undergoing division, in which individual chromosomes could not be distinguished. Hence, no definite statement can be made at this time as to chromosome number or behavior of chromosomes during meiosis.

By the time the original fusion nucleus has given rise to four nuclei, or soon thereafter, the germ tube has begun to branch. Without any noticeable rest period, the nuclei continue to divide. One or more nuclei migrate into the different branches and nuclear division continues as the branches grow. Up to this point few or no septa have formed. Soon, however, in one or more hyphae thick, transparent septa may appear and a change in the method of growth in these hyphae then takes place (Figs. 1, B, and 5, H, I, J, M). The hyphae in which this type of septation has occurred continue to grow and form branches, but the branching is of a different type. The new branches always arise adjacent to a septum and may curl backward or grow in almost any direction. Septa form at short intervals in the curled

branches and more new branches form and curl in a characteristic manner, producing a type of growth that is easy to distinguish from that of colonies with radiating hyphae. As this type of growth continues the septa become greatly thickened. Part of the thick, transparent septum appears to dissolve, so that the mycelium, when disturbed, breaks up into short fragments, each consisting of a single cell or a few loosely attached cells (Figs. 1, B, 2, and 6, B-J).

With this change of growth habit there occurs also a change in the character of the nucleus. The cells of the new type of mycelium are typically uninucleate, but the nuclei are much larger than the haploid nuclei in the



FIG. 2. Fragments of diploid (synkaryotic) mycelium showing separation of individual cells by dissolution of the thick hyaline septa. Each cell contains a single large nucleus believed to be diploid. The nuclei vary in shape but are most frequently oblong. Approx. 1200 \times .

young germ tube. The nucleus is usually oblong rather than spherical, its longest diameter being greater than the diameter of the mycelium. Its oblong shape, therefore, may be the result of compression (Figs. 1, B, 2, and 6, B-J). An occasional cell of this mycelium may be observed with two nuclei, often very close together (Fig. 5, J). In such cells the nucleus is assumed to have just completed division.

These large nuclei stain very heavily and, although in certain individual nuclei chromosome-like bodies could be detected, no definite number could be found with sufficient consistency to justify an unqualified statement as to chromosome content. All circumstantial evidence, however, indicates that these are diploid nuclei and that a fusion of nuclei of opposite sex occurred just prior to the origin of the fragmenting mycelium. The cells of the fragmenting mycelium, therefore, are assumed to be synkaryotic.

Other branches of the same mycelium may continue to grow in the radiate manner. Although the radiate hyphae eventually become septate, the septa are further apart and much thinner. Also, the septa show no tendency to

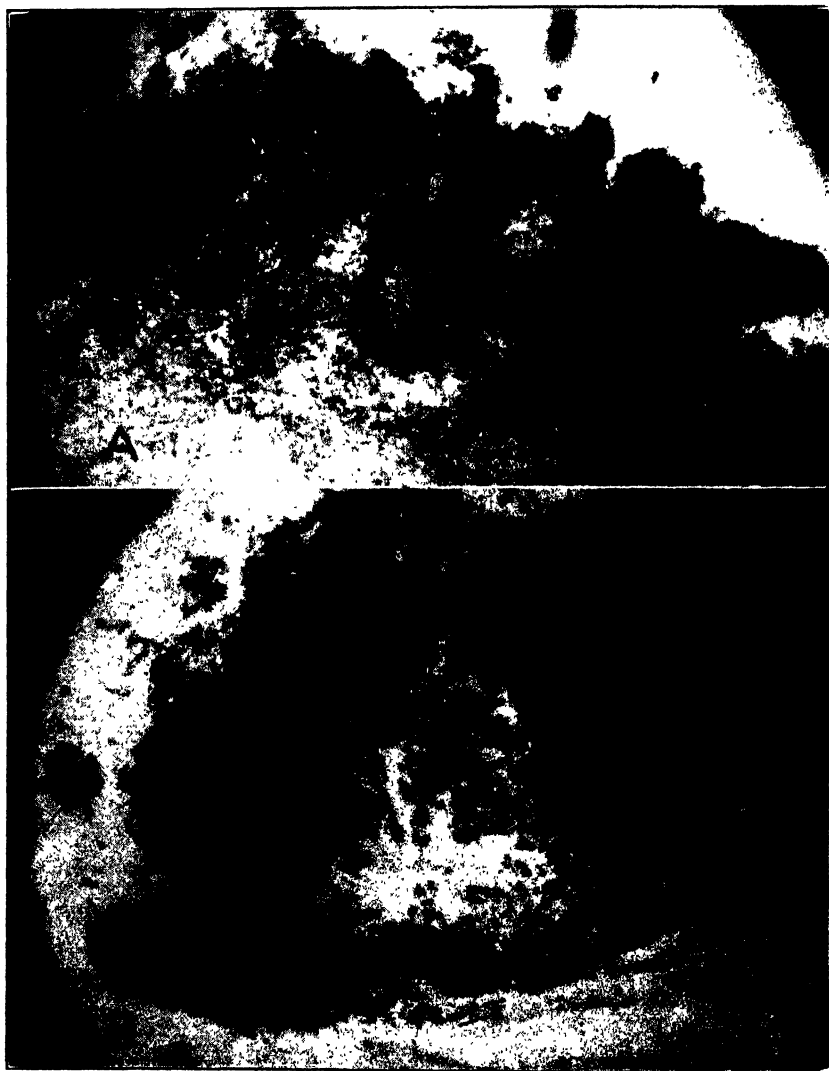


FIG. 3. A. A sector of a vertical cross section of a radiating colony of *Ustilago striiformis* grown on potato-dextrose agar. Near the center of the picture is a young pocket of spores that originated beneath the surface of the colony. Approx. 100 \times . A more highly magnified picture of the spore pocket is shown in figure 4. B. A spore pocket, older and larger than the one shown in A but from the same colony. Undifferentiated mycelial fragments occur in the center of the pocket while mature spores are found at the periphery. Approx. 100 \times .

dissolve and there is no fragmenting of the mycelium. The nuclei of such mycelium are smaller and more nearly spherical (Fig. 6, A). It is believed that these hyphae contain haploid nuclei.

Often fairly large colonies of radiating mycelium may be formed but eventually small pockets of fragmenting mycelium will appear and spores will be formed (Figs. 3 and 4). Spores are never formed directly on radiating hyphae but whenever the fragmenting type of mycelium appears in the colony it, with rare exceptions, proceeds directly to spore formation. This results in pockets of spores that appear to be formed in a centrifugal manner from a central "meristematic" cluster of multiplying mycelial fragments.

In still other cases the change in type of growth may occur very early, sometimes as soon as the germ tube begins to branch (Fig. 5, K), and the entire mycelium will be of the fragmenting type. This behavior may be

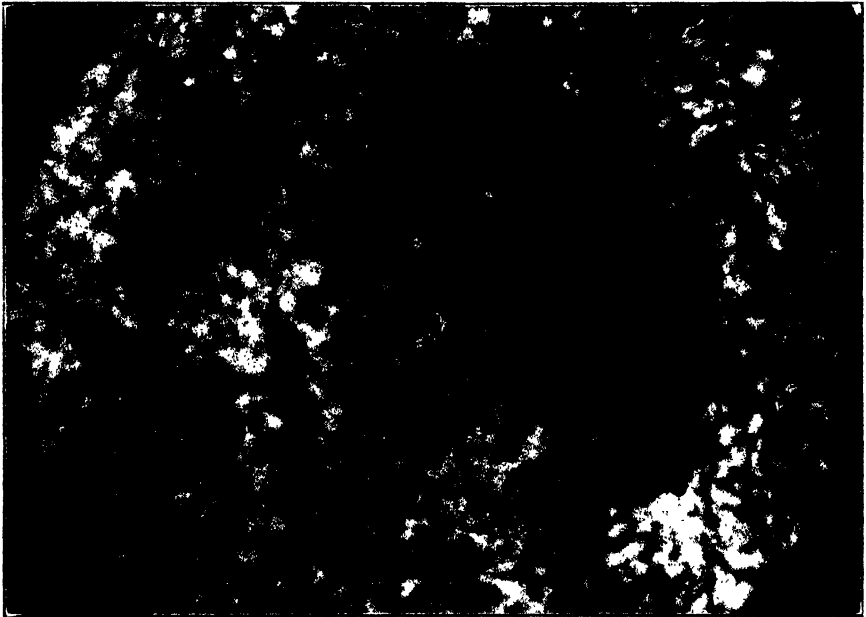


FIG. 4. The spore pocket shown in figure 3, A, magnified about 400 \times . The spores are forming from short mycelial fragments.

due to unusually early karyogamy or, perhaps, to such a failure of the original fusion nucleus to undergo reduction division as was reported by Christensen (3) to occur occasionally in *Ustilago zae*.

The process of spore formation has been studied carefully and no evidence of hyphal fusions has been found. Each cell of a fragmenting mycelium may either grow and form new cells or develop directly into a chlamydospore with no further fusions of nuclei. Various stages in this process of spore formation are shown in figure 6, K-O. The individual hyphal cell enlarges near the middle, becomes shorter and thicker, and gradually acquires a spindle shape. The nucleus enlarges and becomes extremely irregular in outline and somewhat diffuse. As the spore becomes more globular, echinulations appear in the cell wall and the nucleus becomes denser and more regular in outline. At the same time the cell wall takes on a brown color

that increases in intensity as the spore matures. The mature spore is often almost spherical, but many of those formed in artificial culture are slightly

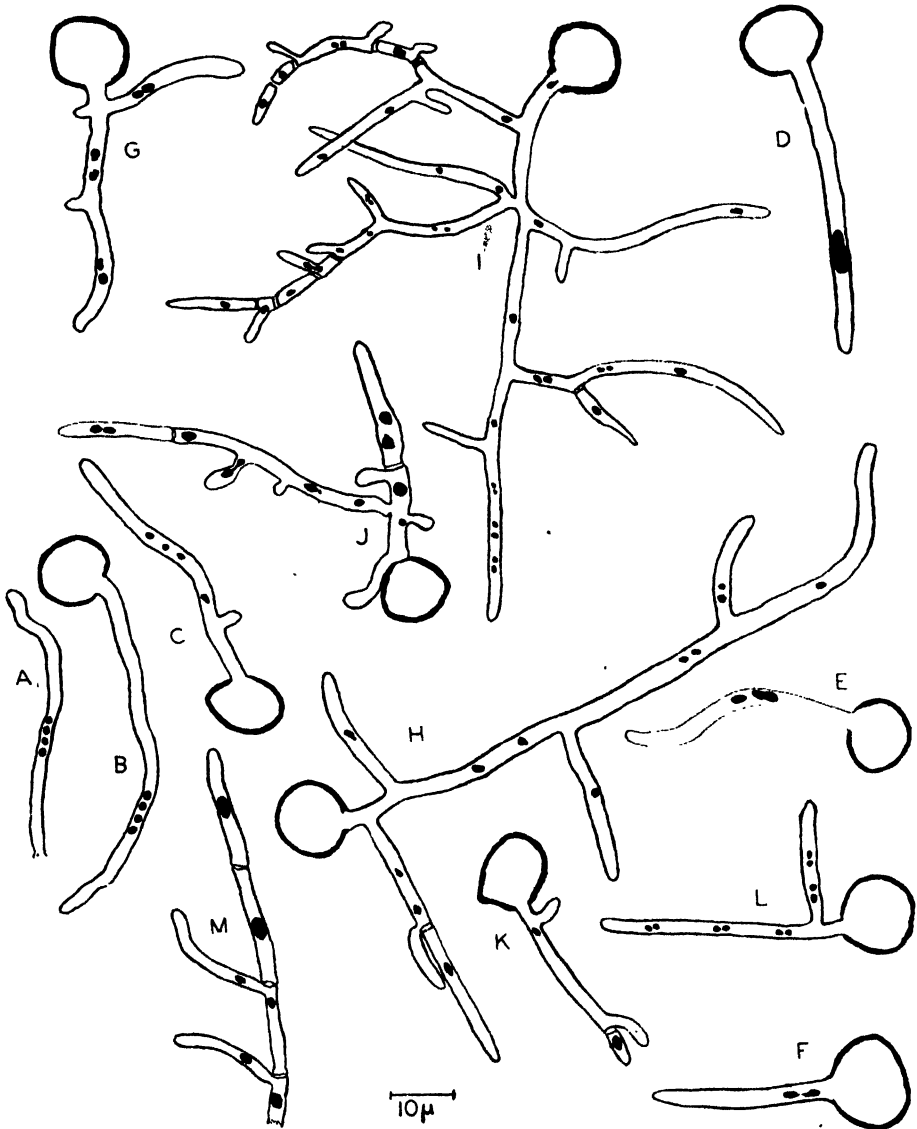


FIG. 5. Germinating spores in various stages of development. A-F, stages in reduction division. G-J, more advanced stages in which further nuclear division has occurred and in which karyogamy is taking place, followed by production of diploid mycelium. K, a form of germination in which the mycelium is of the diploid type from the beginning, indicating absence of reduction division or unusually early karyogamy. L, a germ tube in which there are either 5 pairs of unusually small nuclei or 5 nuclei with two chromosomes each. M, a diploid (synekaryotic) hypha containing four cells, two of which are forming branches and whose nuclei have just undergone division. Approx. 700 \times .

ovoid and some retain the two pointed tips that give them a characteristic lemon shape.

In some cases the mycelium does not all break up into fragments and intercalary spores are formed in chains (Fig. 6, P, Q), but this is not the typical method.

Certain cultures of the fragmenting-mycelium type have never formed spores although they have been cultured on agar for more than two years.

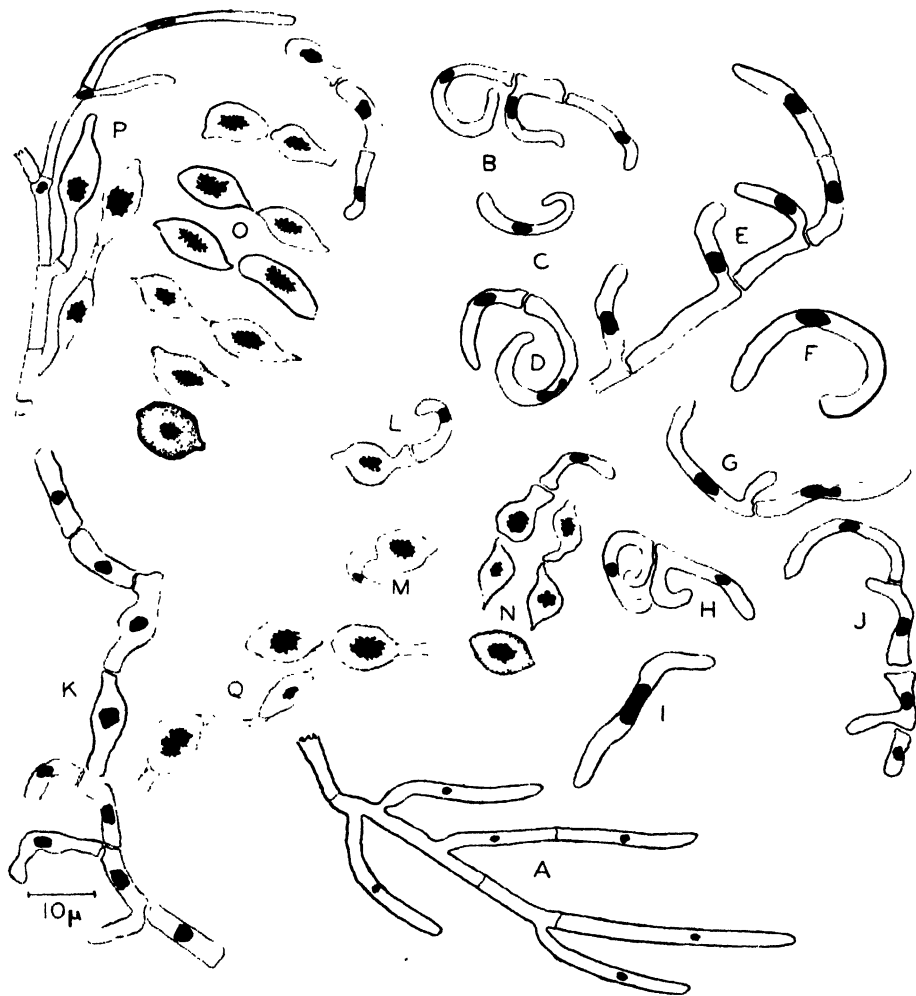


FIG. 6. Haploid and diploid (syngamyotic) mycelia and various stages in spore formation from syngamyotic cells. A, a branched hypha of typical haploid non-fragmenting mycelium with small nuclei and thin septa. B-K, various types of diploid (syngamyotic) hyphae and mycelial segments with cells each containing a single large diploid nucleus. L-Q, various stages in the transformation of syngamyotic cells into chlamydospores. Approx. 700 \times .

No significant differences in their mycelia or manner of growth have been observed. Nuclear stains show that the mycelial fragments in the non-sporulating culture have the same type of large oblong nucleus, but the cells continue to grow vegetatively and do not form spores. No satisfactory explanation for their failure to sporulate can be offered.

DISCUSSION

The cytology of this fungus is different from that of any smut previously described. Since sporidia are not formed and there is no evidence of hyphal fusion, it must be assumed that the fungus is normally homothallic. However, since apparently haploid mycelium is formed, it is possible that by proper manipulation hyphae of opposite sex could be separated. Circumstantial evidence indicates that reduction division occurs following germination and that there is normally a partial recombination of haploid nuclei, presumably of opposite sex, in the branching germ tube. Karyogamy apparently takes place immediately following the recombination, although it is not always possible to distinguish between nuclear division and nuclear fusions in this stage of development. There is no distinct dikaryophase. There is a multiplication of the diploid nucleus and extensive growth of sinkaryotic mycelium preceding spore formation. In so far as the writers know, these two phenomena are not known in any other smut.

It has not been possible to determine what cytological processes take place when the fungus, after growing for a considerable time as a haploid radiate mycelium, changes over to the fragmenting type at localized points in the colony. It could be that there is a delayed pairing and fusion of nuclei of opposite sex, possibly through fusion of adjacent hyphae. No such fusions have been observed, but occasional fusions of this type would not be easy to detect. On the other hand there may be spore formation from bisexual haploid nuclei as reported by Hanna (6, 7) in *Coprinus lagopus*, by Harder (8) in *Coprinus sterquilinus*, and by Winge and Laustsen (16) in *Saccharomyces ellipsoides*. Although no definitely dikaryotic mycelium has been seen, it must be remembered that parallel conjugate division is not the rule in smut fungi and occasional binucleate hyphae might be overlooked.

The cytology and life history of this smut are so strikingly different from those of other smuts generally classified as physiologic forms of *Ustilago striiformis* that one seems justified in questioning its specific identity. The authors consider it unwise, however, to attempt any changes in nomenclature until more extensive comparative studies have been made.

Not only is the cytology of this smut unique for *Ustilago striiformis*, but it also departs widely from the usual concepts of the cytology of smuts in general. The smut apparently is one of the few truly homothallic smuts and, in so far as the authors have been able to learn, no other smut and few fungi are known in which there is a sinkaryotic vegetative stage.

Blizzard (1) found no fusion of sporidia or of saprophytic mycelium of *Urocystis cepulae*. In the host plant the vegetative mycelium was uninucleate until just before sporulation, when binucleate cells appeared without any evidence of hyphal fusion. This has been interpreted (12) as a possible homothallic species.

Graphiola phoenicis probably is homothallic, since, according to Killian (11), its short dikaryophase is not the result of cell fusion. It is possible that *Ustilago ischaemi* is homothallic, since, according to Boss (2), it forms

chlamydospores from uninucleate cells. However, since neither reduction division nor fusion of nuclei were demonstrated for this fungus its condition is subject to other interpretations. [See Kniep (12) and Sampson (14).]

Sampson (14), in reviewing the cytology of the smut fungi, recognized their extreme variability and pointed out how previously accepted concepts were revised as more species were studied. Apparently the present study calls for still further revisions of our concepts of smut cytology.

SUMMARY

Ustilago striiformis from *Poa pratensis* completes its life cycle and forms chlamydospores on agar. A cytological study was made of the fungus in culture and, according to the writers' interpretation of the nuclear behavior, the fungus is normally homothallic. It produces both haploid and diploid (syngamyotic) vegetative mycelium and it has no true dikaryophase. The chlamydospore contains a large nucleus, apparently diploid, that appears to undergo reduction division soon after spore germination. The germinating spore does not form a true promycelium but produces a branched germ tube of indeterminate growth. Sporidia are not produced and hyphal fusions have not been observed. A partial reassortment of nuclei takes place in the branching germ tube or resulting mycelium, and karyogamy occurs without an intermediate dikaryophase. The syngamyotic cells multiply vegetatively forming a characteristic fragmenting mycelium readily distinguishable from the radiating haploid mycelium. The diploid syngamyotic mycelium in its typical form of growth breaks up readily into short fragments, each cell of which contains a single large apparently diploid nucleus. Each cell of the fragmenting syngamyotic mycelium may be transformed directly into a chlamydospore or it may divide to form new syngamyotic cells.

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FUNGISTATIC ACTION OF DIPHENYL ON SOME FRUIT AND VEGETABLE PATHOGENS¹

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In a study of the fungistatic action of diphenyl ($C_6H_5 \cdot C_6H_5$) vapor on citrus fruit pathogens in pure culture there was a wide range of tolerance to this chemical by different fungi.⁴ The growth of some of the most serious citrus pathogens was almost completely inhibited by the diphenyl vapor, some were moderately suppressed, and others grew at practically the normal rate at 40° and 70° F. These studies indicated that further tests with other fruit and vegetable pathogens might be worthwhile since the fungistatic action of diphenyl has been successfully utilized as a means of controlling decay in experimental and commercial shipments of citrus fruit.

The use of diphenyl for controlling the development of decay in packaged commodities is greatly limited because the odor of this chemical is taken up and retained by the cuticle and waxy peel of many products such as apples, pears, peaches, tomatoes, cucumbers, etc. However, the fact that citrus fruits do not long retain the odor after exposure to the air suggested the possibility that certain other fruits and vegetables might not retain the odor. If so, development of decay in such products during transit and marketing might be controlled by diphenyl impregnated wraps, pads, trays, or other containers, provided the pathogenic organisms of such products are satisfactorily controlled by diphenyl vapor. Previous studies showed that diphenyl vapor not only checks vegetative growth of many fungi but also prevents normal spore formation in practically all species of fungi tested. This fungistatic agent therefore should be of considerable aid in controlling the spread of decay by contact and by preventing inoculation of other specimens with spores. It would not be expected, however, that diphenyl would prevent decay in fruit or vegetable products in which the pathogen had already become established previous to packaging.

The chief purpose of the present investigation was to determine the effects of diphenyl vapor on the growth of important fruit and vegetable pathogens found on the market.

Studies were made on 52 organisms representing 35 genera of fungi that cause decay of fruits and vegetables during transit, storage, and marketing. The growth of each pathogen was determined by measuring the diameter of 10 or more colonies grown on potato-dextrose agar in 10-cm. Petri dishes.

¹ Investigation conducted by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, in cooperation with the Department of Botany at the University of Chicago.

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⁴ Ramsey, G. B., M. A. Smith, and B. C. Heiberg. Fungistatic action of diphenyl on citrus fruit pathogens. *Bot. Gaz.* 106: 74-83. 1944.

TABLE 1.—Growth of fruit and vegetable pathogens on potato-dextrose agar plates at room temperature when exposed to diphenyl vapor

Organism	Diameter* of colonies in mm.			
	Control		Diphenyl	
	3 days	7 days	3 days	7 days
<i>Alternaria citri</i> Ell. & Pierce	35	76	20	46
<i>A. radicina</i> Meier, Drechs. & Eddy	25	41	7	11
<i>A. solani</i> (Ell. & G. Martin) L. R. Jones & Grout	31	71	8	16
<i>A. tomato</i> (Cke.) G. F. Weber	33	80	14	31
<i>Aspergillus</i> sp. from nectarines	23	45	0	T
<i>Botrytis allii</i> Munn	26	80	0	0
<i>B. cinerea</i> Pers.	54	80	0	0
<i>Cephalosporium</i> sp. from celery	6	17	T	10
<i>Cladosporium cucumerinum</i> Ell. & Arth.	17	34	11	14
<i>C. herbarum</i> Lk.	28	38	T	7
<i>Colletotrichum circinans</i> (Berk.) Vogl.	20	50	10	22
<i>C. gloeosporioides</i> Penz.	32	74	16	39
<i>Coryneum beijerinckii</i> Oud.	14	28	0	T
<i>Diplodia natalensis</i> P. Evans	72	80	0	T
<i>Dothiorhiza</i> sp. from Avocado	15	80	0	T
<i>Endoconidiophora fimbriata</i> (Ell. & Halst.) Davidson	16	42	7	15
<i>E. paradoxa</i> (Dade) Davidson	80	80	0	T
<i>Fusarium scirpi</i> Lambotte & Fautr.	18	36	T	12
<i>Gloeosporium perennans</i> Zeller & Childs	10	29	8	18
<i>Glomerella cingulata</i> (Ston.) Spauld. & Schrenk	26	74	10	32
<i>Macrophomina phaseoli</i> (Mauhl.) Ashby	80	80	0	T
<i>Macrosporium</i> sp. from tomato	19	51	5	15
<i>Melanconium</i> sp. from tomato	13	33	0	T
<i>Melanospora</i> sp. from potato	32	75	18	58
<i>Monilinia fructicola</i> (Wint.) Honey	16	55	0	0
<i>M. laxa</i> (Aderh. & Ruhl.) Honey	15	48	0	0
<i>Oospora lactis parasitica</i> Pritchard & Porte	17	34	17	31
<i>Penicillium digitatum</i> Sacc.	23	63	0	T
<i>P. expansum</i> Lk. ex Thom	35	47	0	0
<i>P. italicum</i> Wehmer	28	80	T	T
<i>Pestalotia</i> sp. from pineapple	35	80	T	19
<i>Pellicularia microsclerotia</i> G. F. Weber	30	70	0	T
<i>Phoma betae</i> (Oud.) Frank	26	71	T	8
<i>P. sp.</i> from apple	28	76	0	6
<i>P. destructiva</i> Plowr.	21	57	0	10
<i>Phomopsis citri</i> Fawc.	20	57	T	12
<i>P. vexans</i> (Sacc. & Syd.) Harter	43	79	T	11
<i>Phytophthora citrophthora</i> R. E. Sm. & E. H. Sm.	23	57	27	80
<i>Phyllosticta strominella</i> Bres.	29	72	0	8
<i>Pleospora lycopersici</i> El. & Em. Marchal	14	56	0	T
<i>Pythium debaryanum</i> Hesse	80	80	80	80
<i>Rhizoctonia solani</i> Kuehn	80	80	T	8
<i>Rhizopus nigricans</i> Tr.	80	80	0	T
<i>R. sp.</i> (no. 3747) from peanuts	80	80	80	80
<i>Sclerotinia intermedia</i> Ramsey	4	55	0	0
<i>S. minor</i> Jagger	7	80	0	T
<i>S. sclerotiorum</i> (Lib.) DBy	35	80	T	8
<i>Sclerotium rolfsii</i> Sacc.	43	80	T	T
<i>Septoria citri</i> Pass.	7	12	T	T
<i>S. lycopersici</i> Speg.	0	10	0	8
<i>Synecephalastrum</i> sp. from peanut	68	80	0	0
<i>Trichoderma viride</i> Fr.	68	80	17	80

* 80 = full plate; T = trace of growth.

For each organism a set of 10 plates was poured. Two plantings from vigorously growing pure cultures were made on opposite sides half way from the center to the edge of each plate. Five plates were held as controls and in the center of the remaining 5 plates was introduced approximately 60 mg. of diphenyl crystals. The plates were held at room temperature (68–72° F.) for one week and the diameters of the colonies were measured 1, 3, 5, and 7 days after inoculation. The average diameters of 10 or more colonies on the third and seventh days are shown in table 1. At the end of one week the diphenyl crystals were removed from several of the plates in each test to determine whether organisms whose growth had been suppressed would recover. The vapor from 60 mg. of diphenyl crystals did not prove fungicidal to any organism tested. Colonies of each fungus recovered in a few days and normal growth was resumed.

Although accurate comparisons of growth rate between the species of fungi listed in table 1 cannot be made because the cultures were not all made at the same time or grown under identical conditions, the table shows a wide variation in tolerance to diphenyl vapor.

The characteristic growth of the colonies of most fungi suppressed by this chemical is a small, compact mass of distorted, knobby hyphae growing over the square of inoculum and in some cases spreading slightly onto the agar. Organisms that are only slightly checked by the diphenyl vapor show varying degrees of distortion and abnormal growth of the hyphae. Some develop giant swollen cells; others have short head-like strands of cells, and still others lay down numerous cross walls and the hyphae branch excessively. Most organisms do not produce conidia or other fruiting bodies. *Alternaria radicina*, *Pestalotia* sp. and *Cladosporium cucumerinum* were the only organisms, among those suppressed by the vapor, that developed spores, and these spores were abnormal. *Cephalosporium* sp., which was moderately well controlled, developed normal spores in the presence of diphenyl vapor.

Trichoderma viride, *Pythium debaryanum*, *Phytophthora citrophthora*, *Oospora lactis parasitica*, and *Rhizopus* sp. (No. 3747) produced normal hyphae and spores in the presence of diphenyl vapor. *Rhizopus* sp. (No. 3747), isolated from peanuts, a high temperature organism, was suppressed in growth only slightly by diphenyl whereas a good control of *Rhizopus nigricans* was obtained. The effect on the peanut organism was a suppression in luxuriance of growth and in the number of mature sporangia developed. After three days the control plates were full of mature black sporangia while the colonies in the diphenyl plates remained white, developing only a few black sporangia around the edge of the plates.

In order to determine the least amount of diphenyl crystals needed in a 10-cm. plate to check the growth of very sensitive organisms such as *Diplodia natalensis* and *Rhizopus nigricans* and of a moderately tolerant organism such as *Alternaria radicina*, plates were inoculated with each organism in the usual manner. Into one set of plates 1 mg. of diphenyl crystals was

introduced and in other sets 3.5, 5, 10, 15, and 20 mg. per plate were used, respectively. Little or no control of the organisms was obtained by the use of 1 or 3.5 mg. of diphenyl. However, *D. natalensis*, the most sensitive of the organisms, was controlled by 5 mg., producing only a trace of growth within a week. *R. nigricans* was usually controlled by 10 mg. of diphenyl whereas *A. radicina* produced a moderate growth with this amount of diphenyl. This species of *Alternaria* produced practically normal spores with 3.5, 5, and 10 mg. of diphenyl, but with 15 and 20 mg. of the chemical the spores developed were abnormal.

Since diphenyl is moderately fungistatic to a number of organisms and is effective in stopping growth of several important pathogens, there is a possibility of using this chemical for control of decay in some fruits and vegetables. However, further tests will be necessary to determine what products will not absorb the diphenyl odor and to establish the practicability of wrapping such commodities in diphenyl-treated paper or of using other types of treated packages. According to the data obtained so far, successful control of decay is most likely in fruits and vegetables that are affected by certain common decay-producing species of *Aspergillus*, *Botrytis*, *Coryneum*, *Diplodia*, *Dothiorella*, *Endoconidiophora*, *Macrophomina*, *Melanconium*, *Monilinia*, *Pellicularia*, *Penicillium*, *Phoma*, *Phomopsis*, *Phyllosticta*, *Pleospora*, *Rhizopus*, *Sclerotinia*, *Sclerotium*, and *Septoria*. Unfortunately the soft rot bacteria (*Erwinia carotorora* group) pathogenic to most vegetable crops are not held in check by diphenyl vapor.

During this investigation it was found that the differential fungistatic action of diphenyl could be utilized in general laboratory culture work to eliminate some of the more common contaminants. For example, *Rhizopus*, *Penicillium*, and *Neurospora* contaminations may be eliminated from many cultures of other fungi by plating the contaminated culture and adding 60 mg. of diphenyl. Within a week or ten days many fungi will develop colonies large enough to permit transplants from the margin that are free from these contaminants. This method of purifying contaminated cultures of fungi has been used successfully to recover many organisms such as *Alternaria* from *Neurospora*, *Colletotrichum* from *Aspergillus*, *Endoconidiophora* from *Rhizopus nigricans*, *Macrosporium* from *Penicillium*, etc. A study of table 1 will indicate the probable success of separating any one fungus from the others by this method. However, variations in the quantity of diphenyl used and also in length of time the cultures are held may permit separation of some fungi which have a somewhat similar degree of tolerance. For example neither *Botrytis cinerea* nor *Neurospora* sp. grew during a week in plate cultures with 50 mg. of diphenyl, but if a mixed culture of these fungi was held for three weeks the *Botrytis* had grown enough to permit a pure transfer of this organism and thus to eliminate the *Neurospora*.

Since diphenyl has never been fungicidal to any of the organisms tested and transfers of colonies from plates containing this chemical have subsequently grown in a normal manner, it appears that the new technique may be used with safety in separating mixed cultures of fungi.

SUMMARY

In 52 fruit and vegetable pathogens grown on plate culture in the presence of vapor from diphenyl crystals there was a wide variation in degree of tolerance to this chemical. Among the organisms tested, those whose growth was best controlled were species of *Aspergillus*, *Botrytis*, *Coryneum*, *Diplodia*, *Dothiorella*, *Endoconidiophora*, *Macrophomina*, *Melanconium*, *Monilinia*, *Pellicularia*, *Penicillium*, *Phoma*, *Phomopsis*, *Phyllosticta*, *Pleospora*, *Rhizoctonia*, *Rhizopus*, *Sclerotinia*, *Sclerotium*, and *Septoria*.

Diphenyl vapors checked the vegetative growth and prevented normal sporulation of most organisms, but none of the fungi tested was killed.

The use of diphenyl-impregnated wraps, pads, or packages is suggested for controlling diseases of some commodities; but the retention of the odor of diphenyl by many products will make the use of this chemical impracticable.

The differential fungistatic action of diphenyl on many fungi can be utilized as a practical laboratory method of separating mixed cultures. Many cultures of important pathogenic organisms have been cleared of contamination by this method.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,

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AND

DEPARTMENT OF BOTANY,

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PHYTOPATHOLOGICAL NOTES

“*Premunity*.”—In certain publications¹ the term “acquired immunity” has been used to denote the condition that exists in plants that do not exhibit any reaction to a further inoculation with a particular virus after they have once been infected with it or with a closely related virus or strain of virus.

In human and in animal pathology the term “immunity,” or “acquired immunity,” has a different meaning.² It denotes the condition that exists in men or animals that have overcome and are rid of a certain pathogen, and remain non-susceptible to this or to a related pathogen. In other cases, after a first infection, and after health has been partly or apparently restored, the pathogen remains in the body; and nonsusceptibility persists only as long as the pathogen of the first infection is present. As soon as the pathogen has disappeared the host again becomes susceptible to infection by this or a related pathogen. In such cases the term “premunition” is used in French literature.³ It is proposed to use this term, “premunition,” in plant pathology for the condition referred to as “acquired immunity” by Price and others.³—H. M. QUANJER, Landbouwhoogeschool, Instituut voor Phytopathologie, Wageningen, Holland.

Leaf-nematode Infestation of Bird's-nest Fern.—The leaf nematode, *Aphelenchoides fragariae* (Ritzema Bos 1891), causes heavy losses to growers and has ruined the crop of bird's-nest fern (*Asplenium nidus*) in certain years in California. It is the purpose of this paper to point out certain differences between bacterial blight and nematode infestation as they affect the fronds.

The nematodes are introduced with the potting soil (rats-nest material, a natural forest compost) which is the only medium in which bird's-nest ferns are grown commercially in the San Francisco Bay region and which is never sterilized. The symptoms of nematode infestation consist of a slight water-soaking in isolated areas at the base of the fronds; the water-soaking soon disappears, after which the tissues turn a dull brownish-black. The discoloration spreads rapidly under conditions of excessive moisture and relatively high temperature prevailing in the greenhouses. Often several fronds may be involved. Such plants may be killed by the nematodes; large plants survive the nematode infestation although the fronds are badly injured and rendered unsalable.

The nematode-infested fronds of bird's-nest ferns differ from fronds with bacterial leaf blight, caused by *Phytophthora asplenii*, in that affected

¹ Price, W. C. Acquired immunity to ring-spot in Nicotiana. Contr. Boyce Thompson Inst. 4: 359-403. 1932.

² Sergent, E., and L. Perrot. Arch. de l'Inst. Pasteur d'Algerie. 13: 279-319. 1935.

³ Quanjier, H. M. Phytopathologische Terminologie, met speciale bespreking van de begrippen Biotrophie, Premuniteit en Antistoffen. Tijdschrift over Plantenziekten. 48: 1-16. 1942.

tissues are turgid and dull-brownish black, while bacterial blight causes a water-soaked, soft condition of the leaves, the affected tissues being easily crushed when light pressure is applied.

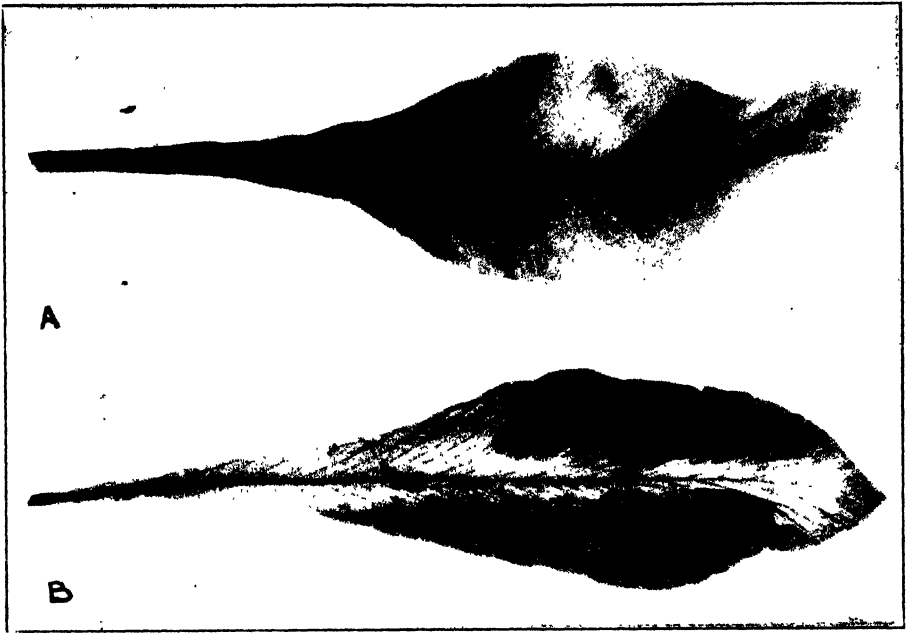


FIG. 1. A. Leaf of bird's nest fern infested with the leaf nematode, *Aphelenchoides fragariae*. B. Leaf of bird's nest fern infested with *Phytonomonas asplenii*.

Control of nematode infestation of bird's-nest ferns has been obtained by steam sterilization of the potting soil, flats, and pots at several nurseries in San Francisco. — PETER A. ARK and C. M. TOMPKINS, Division of Plant Pathology, University of California, Berkeley, California.

More on the Name Ansatospora acerina.—In 1941 the writer¹ announced the discovery of a black rot of celery in cold storages due to *Cercospora cari*, a fungus previously described by Westerdijk and van Luijk² as the cause of anthraenose of caraway. In a more complete description of the celery disease in 1944 the writer³ elevated the pathogenic species to generic rank, giving it the name *Ansatospora macrospora*, chiefly because of a swordlike appendage found on mature spores. This specific name had been applied to the same fungus by Osterwalder⁴ who described its attack on pansies a few months previous to Westerdijk's and Luijk's description. In 1945 Han-

¹ Newhall, A. G. An undescribed storage rot of celery. (Abstr.) *Phytopath.* **31**: 17. 1941.

² Westerdijk, Johanna, and A. van Luijk. Eine Anthraknose des Kümmels (*Caram carvi*). *Meded., Phytopath. Lab. Willie Commelin Scholten, Baarn* **8**: 51-54. 1924.

³ Newhall, A. G. A serious storage rot of celery caused by the fungus *Ansatospora macrospora* n. gen. *Phytopath.* **34**: 92-105. 1944.

⁴ Osterwalder, A. Ueber die durch *Cercospora macrospora* Osterwalder verursachte Blattkrankheit bei den Pensees. *Mitteil. der Thurgauischen Naturf. Gesells.* **25**: 59-80. 1924.

sen and Tompkins⁵ called attention to the fact that in 1880 Hartig⁶ undoubtedly had described the same fungus on seedlings of *Acer pseudoplatanus* in Bavarian tree nurseries and had named it *Cercospora acerina*, and therefore if it deserved generic rank the name should be *Ansatospora acerina* (Hartig) n. comb. The writer not only agrees with this reasoning but in seeking verification has since inoculated the cotyledons of *Acer pseudoplatanus* with a pure culture from celery and obtained infections and sporulation which indicates the identity of the two pathogens.

But because of the war, we have all missed the first paper in which this fungus was correctly described as the type species of a new genus. Paul Neergaard⁷ found a fungus on parsley roots in Copenhagen in 1942 which he named *Centrospora Ohlsenii*. He gave a Latin description, with drawings and measurements which leave no doubt of the identity of the fungus with the writer's description of *Ansatospora macrospora*. However, Neergaard, evidently, like the writer, had not seen Hartig's paper. Priority and the rules of nomenclature dictate the correct name for this fungus as *Centrospora acerina* (Hartig) n. comb.

There are now several synonyms for this fungus. As early as 1896 in the second edition of his book on the diseases of plants, p. 318, Frank⁸ rather arbitrarily changed the name from *Cercospora acerina* Hartig to *Sporidesmium accrinum* (Hartig) Frank. In 1918 Arnaud⁹ again finding it on maple seedlings, called it *Cercosporella acerina* (Hartig) Arn. In his book on the control of diseases and insect pests of ornamentals Pape¹⁰ follows Arnaud's nomenclature while in a similar book by Flachs,¹¹ that of Hartig is used.

Another synonym as well as a new host is of interest. In 1937 Sprague¹² described a fungus causing a leaf spot of *Osmorhiza breviplex* growing along the moist bank of a stream in Benton County, Oregon. He named it *Cercospora praegrandis* on account of the large conidia. Specimens sent by Sprague to C. Chupp have been found by him and the writer to bear conidia identical to those of *Centrospora acerina*. The genus *Osmorhiza* moreover is a relative of the genera *Petroselinum*, *Carum*, and *Apium* on which the fungus has already been described. The list of synonyms for *Centrospora acerina* (Hartig) n. comb. therefore includes the following:

⁵ Hansen, H. N., and C. M. Tompkins. The name of *Ansatospora macrospora*. *Phytopath.* 35: 218-220. 1945.

⁶ Hartig, Robert. Der Ahornkeimlingspilz, *Cercospora acerina* m. Untersuch. Forstbot. Inst. München 1: 58-61. 1880.

⁷ Neergaard, Paul. Mykologische Notizen II. Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, (II) 104: 407-411. 1942.

⁸ Frank, A. B. Krankheiten der Pflanzen. Band. 2. 574 pp. Breslau. 1896.

⁹ Arnaud, S. Le mildou des lilas et la maladie des cotyledons d'érable. *Bull. Soc. Path. Veg. France* 5: 58-60. 1918.

¹⁰ Pape, Heinrich. Die Praxis der Bekämpfung von Krankheiten und Schädlingen der Zierpflanzen. 361 pp. Berlin. 1932.

¹¹ Flachs, Karl. Krankheiten und Parasiten der Zierpflanzen. 558 pp. Stuttgart. 1931.

¹² Sprague, Roderick. New or noteworthy parasitic species of Fungi Imperfecti in Oregon. *Mycologia* 29: 426-433. 1937.

- Cercospora acerina* Hartig. Untersuch. Forstbot. Inst. München **1**: 59. 1880.
- Sporidesmium accrinum* (Hart.) Frank. Krankheiten der Pflanzen **2**: 318. 1896.
- Cercosporella acerina* (Hart.) Arnaud. Bul. Soc. Path. Veg. France **5**: 60. 1918.
- Cercospora macrospora* Ostw. Mitt. Thurgau. Natur. Ges. **25**: 59. 1924.
- Cercospora Cari* Westerdijsk & van Luijk. Meded. Phytopath. Lab. Baarn **8**: 53. 1924.
- Cercospora praegrandis* Sprague. Mycologia **29**: 431. 1937.
- Ansatospora macrospora* (Ostw.) Newhall. Phytopath. **34**: 98. 1944.
- Ansatospora acerina* (Hart.) Hans. & Tompk. Phytopath. **35**: 220. 1945.
- Centrospora Ohlensii* Neergaard. Zentralbl. Bact. Par. u. Infek. **104**: 410. 1942.

Since the genus name *Ansatospora* must be rejected in favor of the prior name *Centrospora* it becomes necessary to change *Ansatospora Bromi* Sprague to *Centrospora bromi* Sprague, if indeed it belongs here rather than in the genus *Ramulispora*. This species occurring on *Bromus rigidus* was first described as *Cercospora Bromi* by Sprague¹³ and later¹⁴ changed to *Ansatospora Bromi* on account of "its peculiar secondary conidia which are attached and down deflected from the first or second basal cell of the conidia."

In reviewing the host range of *Centrospora acerina*, Hartig's statement that it is capable of living as a saprophyte in the soil is brought to mind by the report of Rader¹⁵ who isolated it from muck-grown carrots several times during the winter and spring of 1945 in cold storages in Wayne County, New York, where it has been troublesome on celery for many years. The carrots are believed to have shown no symptoms at the time they were harvested and placed in cold storage, a fact previously recorded by the writer to be the rule in connection with celery. Specimens of badly affected pansies were sent by Mrs. B. R. Rogers from Puyallup, Washington, to Mr. Rader at Cornell in the spring of 1945. Dr. Chupp has a specimen of affected *Viola* from Wrangell, Alaska, collected September 3, 1934, by G. F. Gravatt. Trusecott¹⁶ states the fungus can attack the roots of beets, rutabagas, and potato tubers. It is noteworthy that maple seedlings and pansies are affected in early spring, that Sprague's collection on *Osmorhiza* was made April 25, that on caraway the infections were severe on seedlings in spring and on seedstalks in the fall, while on celery and carrots the disease does not show until after two or more months in cold storage at 32-33° F. All of this

¹³ Sprague, Roderick. Undescribed species of *Cercosporella* and *Cercospora* on certain grasses in Oregon and Washington. Mycologia **29**: 199-206. 1937.

¹⁴ Sprague, Roderick. Additions to the Fungi Imperfecti on grasses in the United States. Mycologia **38**: 52-64. 1946.

¹⁵ Rader, Wm. E. *Ansatospora acerina* found causing decay of stored carrots in Wayne County, New York. U. S. Dept. Agr., Plant Dis. Repr. **29**: 522. 1945.

¹⁶ Trusecott, J. H. L. A storage rot of celery caused by *Ansatospora macrospora* (Osterw.) Newhall. Can. Jour. Res. (C) **22**: 290-304. 1944.

clearly shows the fungus to be a low temperature pathogen with a rather wide host range, unlike the leaf-spotting members of the genus *Cercospora*.

From an examination of several exsiccati specimens in the United States labeled *Cercospora acerina* (Hartig) it is apparent that some are true *Cercosporas*, and should be assigned either to *C. negundinis* E. and E. or to *C. acericola* Woronichin, both of which occur on fully expanded leaves in the summer months rather than on cotyledons in the early spring. This would seem to be a helpful gross diagnostic character. Specimens on *Acer* cotyledons labeled *C. acerina* Hartig are more likely labeled correctly except that the fungus now becomes *Centrospora acerina* (Hartig) n. comb.

The ability of this fungus to live in the soil, to attack a rather wide variety of hosts, and to grow at low temperatures suggest that it might become a serious pathogen were it not for the fact that the conidia are exacting in the conditions requisite for their formation and they are not very long-lived. The fungus seems to be most troublesome in situations where high humidities, high soil moistures, and temperatures below 65° F. prevail over long periods, in other words in a maritime climate.—A. G. NEWHALL, Department of Plant Pathology, Cornell University, Ithaca, New York.

SPINDLING OR HAIR SPROUT OF POTATO

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S. J. FAIRCHILD

(Accepted for publication June 16, 1946)

When potato tubers (*Solanum tuberosum* L.) produce sprouts that are slenderer and weaker than normal, the disorder is generally referred to as spindling sprout or hair sprout, depending upon the severity of the symptom. Although various causes, especially a virus (9, 25), have been suggested during the past 30 or 40 years, the nature of much of the spindling sprout that occurs today remains obscure (12).

In 1942, an outbreak occurred in plantings of the White Rose variety of potato along the coast of California. The findings reported here on the nature of spindling sprout are a result of investigations initiated then. They apply, however, only to the disease as it has occurred along the California coast, since no study has been made of spindling-sprout tubers from other regions.

Economic loss from spindling sprout results from the failure of affected planting stock to give good stands, and the failure of plants from affected stock to produce satisfactory yields.

SYMPTOMS

Affected uncut tubers sprout prematurely, though seldom—in California—immediately after harvest. Most of the eyes of such tubers produce sprouts varying from slightly longer and more slender than normal to threadlike growths (Fig. 1) up to a foot or more in length. Tubers about the size of a pea or smaller may be formed at the tip or elsewhere on the spindly sprout. Severely affected tubers often fail to sprout. Diseased tubers are somewhat brittle and are low in starch and specific gravity. Similarly affected tubers in Minnesota are reported (11) to be high in reducing sugar and total sugar and low in dry weight. The differences are not sufficiently great, however, to permit separation of affected and healthy tubers with certainty before sprouting.

EVIDENCE AS TO THE CAUSE OF SPINDLING SPROUT

Spindling sprout has been attributed, at least tentatively, to hot, dry weather of the preceding growing season, Fusarium wilt infection, infection by the leaf roll, witches' broom, aster yellows or some other virus, faulty storage conditions, purple top, or some other less tangible cause (2, 3, 5, 6, 8, 13, 14, 15, 16, 17, 18, 19, 21, 28, 29).

Several workers (11, 17, 20) have used grafting as a means to determine whether spindling sprout is caused by a virus. The results have been, on the whole, adverse to such a conclusion.

However, in order to test further the hypothesis that the spindling sprout

in California might be caused by a virus, several attempts were made to transmit the disease by grafting. Sprouted eyes from healthy tubers were replaced with eyes from sprouted diseased tubers, and vice versa. Also, spindling sprouts on diseased tubers were inarched to normal sprouts on healthy tubers. In none of these experiments did any change develop in the grafted parts or in the sprouts from undisturbed eyes of tubers.

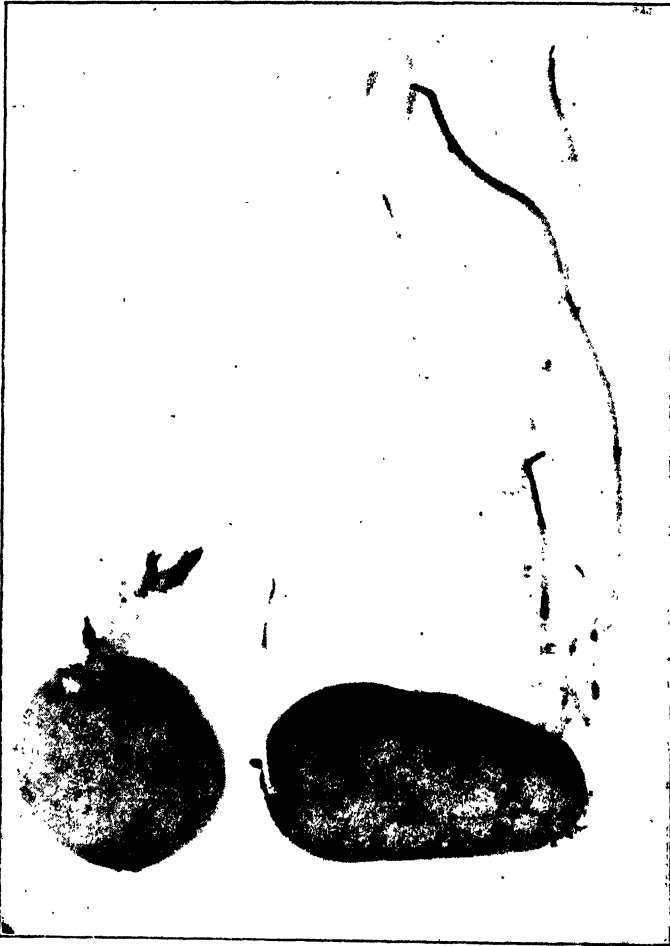


FIG. 1. Sprouted tubers from plant not infested (left) and infested (right) with psyllids in the greenhouse.

Diseased and healthy tubers were planted in greenhouse and field to determine if virus symptoms could be detected in the plants grown from such seed or in their progenies. A commercial seed stock of White Rose was secured which was known to contain a considerable number of affected tubers. Uncut tubers were held at room temperature until sprouting was well advanced, then separated into lots with normal and spindling sprouts. The tubers were cut into seed pieces with one sprout each and 20 hills of

each lot were planted in the field near Berkeley. All sprouts were held upright and were protected from damage while the soil was placed around them.

As soon as the spindling sprouts started to produce their own roots they began to lose their spindly characteristic. The apical portion of the sprouts thickened and soon after emergence the spindling sprouts closely resembled healthy sprouts. They were slower than normal sprouts, however, to appear above ground. This was in striking contrast to the growth of the sprouts in the laboratory where roots were absent and all nutrition was supplied by the seed piece. Without functional roots the spindling sprouts maintained a rather uniform diameter whether the growth was 2 inches or 2 feet in length. With functional roots, the spindling sprout, now an underground stem, eventually ceased to behave as a spindling sprout.

The foliage from these 2 lots of tubers was in every way comparable in appearance and in freedom from virus symptoms. The yield from the spindling sprout seed was about one-half that from the healthy seed. Tubers from these two lots were harvested and the progeny from each plant kept separate for a sprouting test. All progeny sprouted normally irrespective of whether they were derived originally from spindling sprout or normal tubers. These tubers were then replanted. The resulting plants were indistinguishable in appearance or in yield.

A smaller test of the same kind carried out in the greenhouse and two other field tests later gave similar results. For example, in one field test the yield from 10 blocks of normal seed potatoes containing an average of 18 hills per block was 266.5 pounds, whereas that from 10 blocks of spindling-sprout seed potatoes containing an average of 14 hills per block was 127.5 pounds. The average yield per hill was 1.5 pounds for normal seed potatoes and 0.8 pounds for spindling-sprout seed potatoes. In comparing these yields the reduction in stand obtained from the spindling-sprout seed and the resulting difference in spacings between hills must be taken into account.

All evidence gained from these tests supports the view that the spindling sprout studied is not caused by a virus. Spindling sprout was not transmitted to the progeny of plants grown from spindling-sprout seed, nor artificially by grafting. Furthermore, these tests showed that spindling sprout is a temporarily abnormal condition, recovery from which begins as soon as the diseased sprouts strike roots into the soil.

Toxin Indicated as a Cause. Since all available evidence was against a virus cause for the 1942 outbreak of spindling sprout in California and since no organism was found consistently associated with affected tubers, a toxic substance in the tuber itself was suspected. This hypothesis immediately suggested a possible relationship of spindling sprout to psyllid yellows, caused by *Paratrioza cockerelli* Sule.

In their paper on psyllid yellows of potato Richards and Blood (21) state that "tubers seldom reach marketable size and may sprout directly

without going into a rest period." They do not discuss the character of sprouting in tubers from psyllid infested plants, but state that the psyllid yellows disease is not tuber transmitted. Hartman (10) also observed in Wyoming that tubers from psyllid infested plants fail to go through a normal rest period and tend to sprout much earlier than normal tubers. Schaal (24) prepared an extract from crushed psyllids and injected it into potato plants. Not only did he report symptoms of psyllid yellows in the tops of these injected plants but noted that the tubers therefrom developed sprouts that were at first spindling. Schaal also made the incidental observation that psyllid infested plants produce tubers that give rise to normal plants except for spindly stems. That seed tubers from plants with severe vine injury caused by psyllids may develop weak stems and result in poor yields was shown also by Edmundson (6).

Experimentally Induced Spindling Sprout. In two greenhouse tests where a group of potato plants was intentionally infested with psyllids while another group was kept for controls, mild symptoms of spindling sprout were obtained when the tubers harvested from the infested plants had sprouted. Although well over a hundred psyllids were colonized on the infested plants, the work was done in the winter under poor light and even the foliage had very mild symptoms. However, since in these tests the noninfested controls produced only tubers that sprouted normally, the tendency for tubers from psyllid plants to produce spindly sprouts appeared significant. Moreover, a few tubers from this experiment developed typical spindling sprout.

In another experiment, 14 healthy potato plants of the White Rose variety were grown in 5-gallon cans in the greenhouse until tubers 1 to 2 inches long had set. Populations of over a hundred psyllids were at that time established on each of 7 plants. All plants were then moved outdoors into full summer sunlight, the controls being separated from the infested plants by a greenhouse. Typical, severe psyllid yellows symptoms appeared on the infested plants and the controls remained free from infestation.

The tubers were harvested separately from each plant and stored at room temperature until sprouted. Results are illustrated in figure 2. One or more tubers from each of the infested plants developed typical spindling sprout. Some tubers from infested plants never sprouted. Those which put forth sprouts did so well in advance of the tubers from the controls, and from more eyes per tuber. Every tuber from the control plants produced one to several normal sprouts. The resemblance of the spindling sprouts induced in this experiment to those obtained originally from the field or to those illustrated elsewhere (8, 11, 12, 14, 20, 28) is striking.

Before discarding the tubers from this experiment they were cut. Those harvested from psyllid-infested plants showed not only spindling sprout but also an internal browning resembling that reported by Sanford

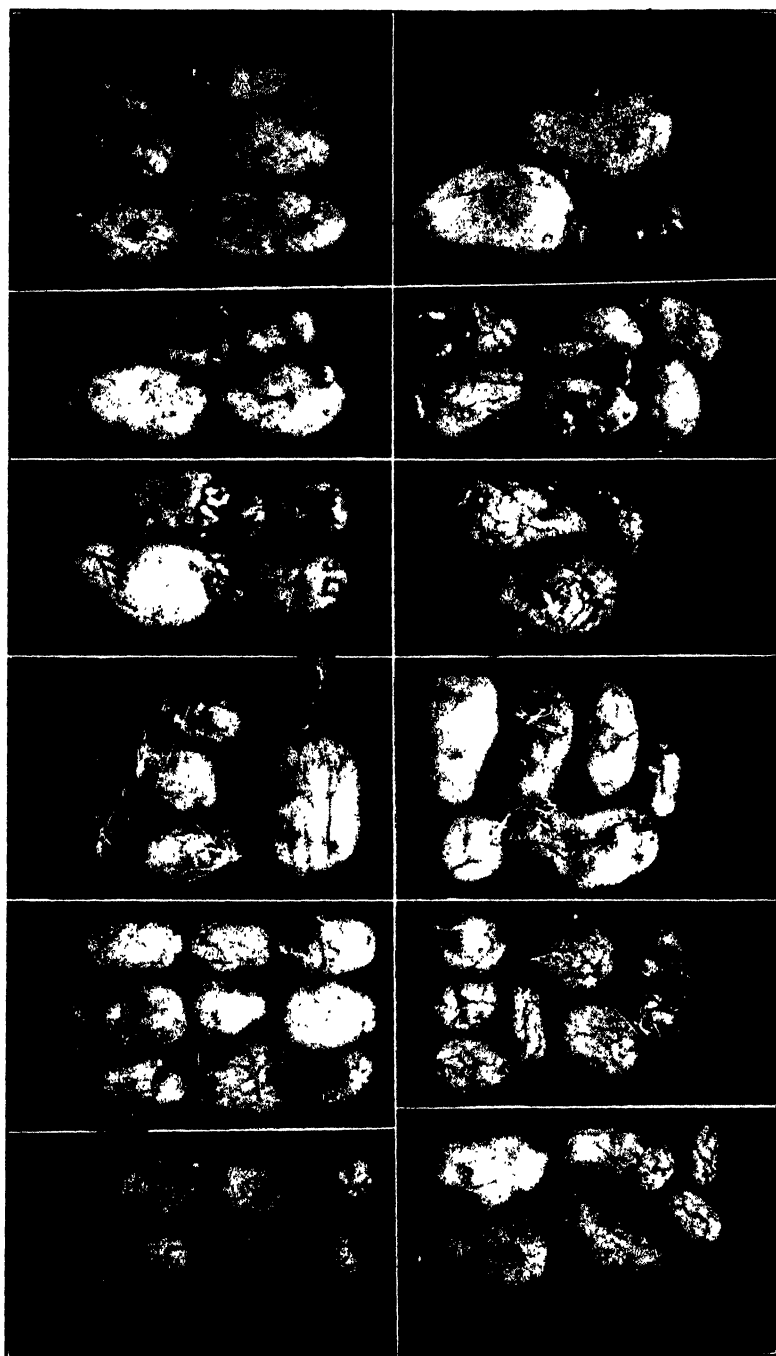


FIG. 2. Sprouted progeny from 6 noninfested plants (top) and 6 plants heavily infested (bottom) with psyllids, under outdoor conditions. Some affected tubers failed to sprout.

(23). These observations (27) confirm the suggestion that an infestation of the potato plant with psyllids may result in an internal necrosis of the tubers.

In another experiment an outdoor planting was made in midsummer and all plants were caged individually before plant emergence. To one group of plants, psyllids were introduced 2 months later. A second group was left noninfested for controls. A third group was infested with psyllids and at the same time the soil was inoculated with *Fusarium oxysporum* f. *tuberosi* Snyder and Hansen. A fourth group was inoculated with the *Fusarium* but not infested with psyllids. The result was that the progeny from all plants infested with psyllids developed typical spindling sprouts whether *Fusarium* was present or not, while all tubers from plants without psyllids sprouted normally.

DISCUSSION

Evidence presented here confirms the work of Schultz (26), Parris and Jones (20), and Harvey *et al.* (11) on one hand, and that of Richards and Blood (21), Hartman (10), and Schaal (24) on the other, that neither spindling sprout nor psyllid yellows is tuber transmitted. No involvement of a virus has been indicated in either case. That it is a toxic principle from the psyllid that produces both the foliage symptoms known as psyllid yellows and the symptom known as spindling sprout is supported by Schaal's (24) experiment in which he injected an aqueous filtrate of crushed psyllids into the potato plant.

Spindling sprout has been sporadic in its appearance, as have severe infestations of psyllids. Psyllids have been reported from Utah, Arizona, New Mexico, California, Washington, Oregon, Idaho, Montana, South Dakota, North Dakota, Kansas, Nebraska, and western Canada (10). Since psyllids are not conspicuous on a potato plant it is possible that a search may reveal their presence in still other potato-growing states. Relatively small numbers of psyllids on plants approaching maturity are probably sufficient in most cases to induce spindling sprout of the tubers.

It is possible that a toxicogenic insect other than psyllids may be responsible for non-virus spindling sprout in those regions where psyllids do not occur on the potato plant. If so, observations on the occurrence of spindling sprout in eastern states (1, 2, 8, 14, 28) would indicate an insect favored by a hot, dry growing season. In Iowa it has been observed (13) that more hair sprout has appeared in seasons immediately following years of severe purple top. This suggests that spindling sprout is a symptom of purple top as well as of psyllid yellows.

The prevention of spindling or hair sprout, where caused by the tomato psyllid, would be the same as that already worked out for psyllid yellows, namely, the frequent applications of sulphur dusts or sprays (4, 10, 22). In fact, recognition that psyllid infestations may result in the formation of spindling sprouts in the progeny provides an additional reason for psyllid control practices in seed potato regions.

SUMMARY

Artificial infestations of potato plants with psyllids under controlled conditions have shown that typical spindling sprout or hair sprout may be a symptom of psyllid yellows, when psyllids are present on plants approaching maturity.

The form of spindling sprout studied is not tuber transmitted, nor is there any evidence of the involvement of a virus.

There is no tendency for sprouts to grow out of the spindling sprout condition as long as all sprout growth is sustained by the tuber. Spindling sprouts that have struck roots into soil begin to grow normally.

The yield from spindling-sprout tubers was approximately one-half that from normal sprouting seed. The second generation from spindling-sprout tubers fully recovered from all spindling-sprout symptoms and yielded normally.

Severely affected tubers may fail to sprout.

Internal necrosis in tubers affected with spindling sprout was observed in one experiment in which the tubers were stored for several months.

The relation between spindling sprout and psyllid yellows makes doubly important the application of control practices against psyllids, especially in areas where seed potatoes are produced.

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BLACK ROT OF MUSCADINE GRAPES¹

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Black rot, a widespread and destructive disease of bunch grapes, is also a common disease of muscadine grapes, *Vitis rotundifolia* Michx. On muscadine grapes it is primarily a disease of the vegetative parts; while on bunch grapes it is primarily a disease of the fruit, mummifying the berries and often destroying a large part of the crop. In addition to differences in symptoms and in amount of damage to the crop the black rot disease of muscadine grapes differs from that of bunch grapes in etiology. The disease as it occurs on muscadine grapes has, however, received only incidental consideration, although a great amount of work has been done on black rot of bunch grapes. Since the muscadine grapes are now becoming of increased economic importance, it seems desirable to present an account of work done during the past four years at the Georgia Experiment Station on the symptoms, etiology, and control of the black rot disease on these grapes and to attempt an estimate of the effect this disease may have upon the production of the crop in the future.

SYMPTOMS

Black rot is common in muscadine vineyards in Georgia throughout the growing season. In fact, from April to late August, it is the only disease of any importance found in these vineyards. Other diseases become prevalent only toward the end of August as the berries approach maturity. Angular leaf spot (3) then appears on the foliage, and bitter rot and various ripe rots develop on the maturing berries. All of these diseases may be easily distinguished from black rot. Nevertheless, because of the superficial resemblance of bitter-rot berry mummies to the berry mummies produced by black rot on bunch grapes, bitter rot and black rot of muscadine grapes have sometimes been confused by growers. The two diseases should be distinguished, however, as black rot usually causes only a scab or canker of muscadine berries, while bitter rot reduces the berries to shriveled black mummies.

On Leaves. The first symptoms of black rot are leaf spots which appear almost invariably each year about four weeks after the shoots begin their annual growth. The spots appear suddenly throughout the vineyard and attain their full size almost as soon as they become evident. They are

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Work on diseases of muscadine grapes was turned over to me in October, 1942, by Dr. K. H. Garren. Dr. F. F. Cowart, Horticulture Department, Georgia Experiment Station, has collaborated on this project to the extent of applying sprays in experiments on control and furnishing data on yields.

I am indebted to Dr. B. B. Higgins, Dr. F. F. Cowart, Dr. K. H. Garren, Georgia Experiment Station, Dr. J. L. Weimer, Bureau of Plant Industry, and Dr. Frederick A. Wolf, Duke University, for their criticisms of the manuscript and to Mr. J. G. Futral for taking the photograph reproduced in figure 3, B.

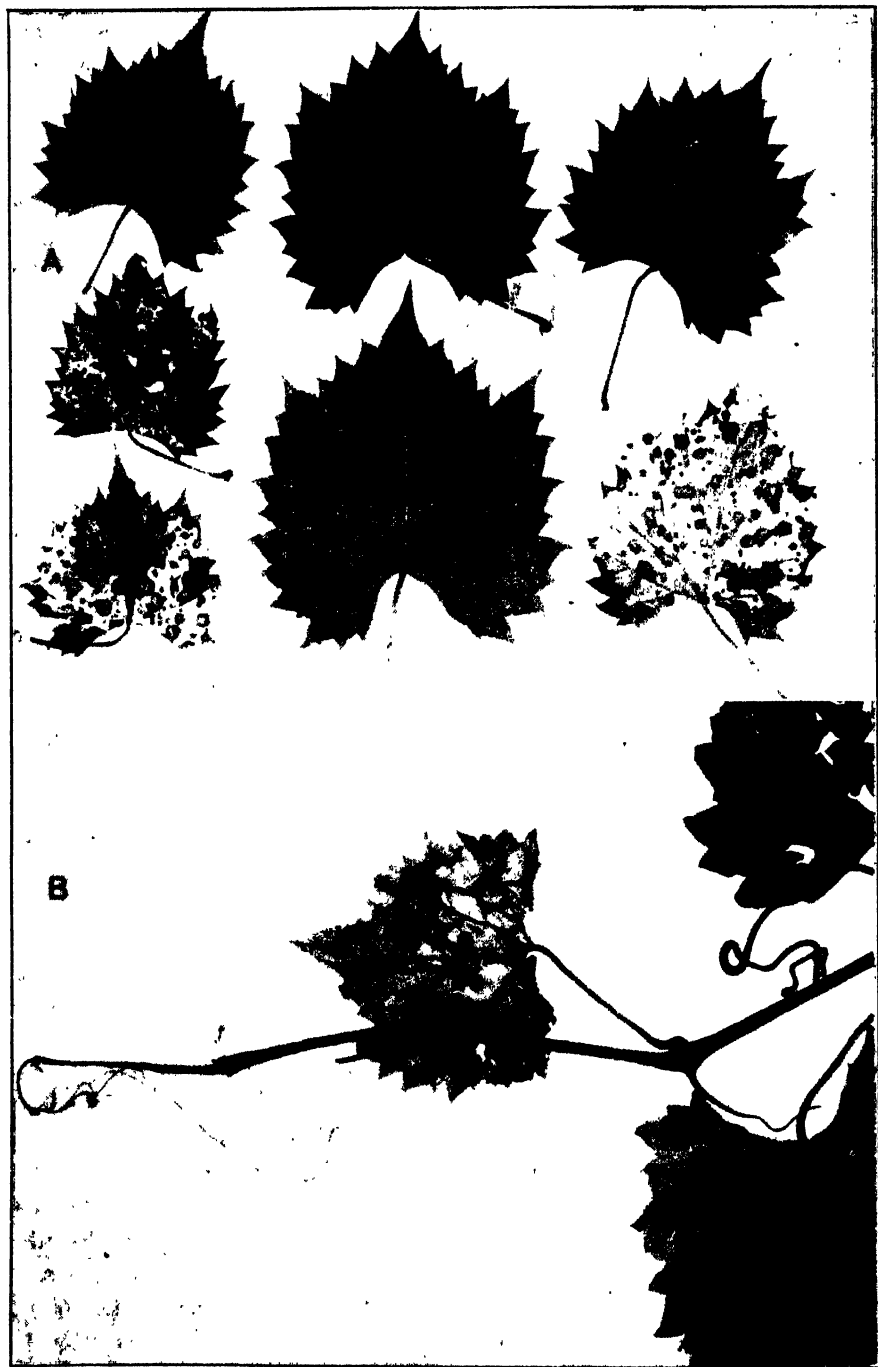


FIG. 1. A. Black-rot spots on leaves of a muscadine grape; upper four leaves from the upper surface, lower three leaves from the lower surface; $\times \frac{1}{2}$. B. Black-rot stem tip blight of a muscadine grape shoot; $\times \frac{1}{2}$.

first noticeable as faint blanched areas in the leaf blade. The green color of the infected tissue fades; and the spots become cream-colored, the color gradually deepening to tan and ultimately on the upper surface to reddish-brown. They are usually bordered by a narrow band of dark brown tissue. Pycnidia develop within two or three days on both surfaces of the leaf. When mature they appear as small black pimples in the dead brown tissue of the spots. The spots are circular and vary in diameter from less than one to 12 mm. (Fig. 1). Large spots may coalesce and kill a large part or all of the leaf blade (Fig. 1). Infection of major veins also may result in the death of large areas of the leaf (Fig. 1). Mature leaves are not infected.

Petioles may become infected at the same time as the leaf blades. Either one to several small, scabby, flat or slightly elevated black lesions or larger, depressed cankers develop in the petioles. Small lesions apparently do not interfere with the functioning of the petiole, but large cankers may girdle the petiole and kill the entire leaf (Fig. 2, A).

Infection of immature leaves continues to occur during periods of favorable weather throughout the season until growth of the vines ceases in late fall. Generally, however, black rot causes little defoliation.

On Stems. Infection of the stems just back of the growing tip occurs throughout the growing season. Either small, black, scabby lesions (Fig. 2, A) or elongated, black, depressed cankers (Fig. 2, B) are produced in the surface of the stem. Numerous cankers sometimes cause a blight of the growing tips of the shoots. The stem becomes blackened and dies, the leaves drop off, and the tip of the stem curves back to form a crook (Fig. 1, B). Stem tip blight is especially common after periods of moist weather in spring. Like the stems, tendrils may be more or less covered with cankers or even killed.

On Flower Clusters. Black rot also affects the flower clusters. Cankers similar to those on the petioles appear on the peduncles and pedicels (Fig. 2, A). Small cankers apparently cause little damage, but more extensive cankers may kill a portion or all of the cluster (Fig. 2, A). Blight occurs chiefly on the first flower clusters produced in the spring. Those formed later in the season are rarely blighted. Moreover, the cankers apparently do not spread in the pedicels and peduncles which survive infection.

On Berries. On most varieties of muscadine grape only one to several small, black, superficial, scabby lesions one to two mm. in diameter are formed in the skin of the berry (Fig. 3, A, row 3, No. 2-5). Occasionally, especially on the Dawn variety, the scabs may coalesce to form a brown to black mosaic-like crust covering a large part of the surface of the berry (Fig. 3, A, row 3, No. 1). Cankers up to 10 mm. in diameter may be produced on the berries of the most susceptible varieties (Fig. 3, A, row 2, No. 6-7). The skin often splits at the periphery of these cankers, exposing a band of brownish corky tissue which forms a border around the canker. The surface of the canker is cracked and fissured and is roughened with embedded pycnidia. Infection takes place on the immature berries from

the time they are set until they approach full size. The lesions do not spread in mature berries, and these dry, superficial scabs and cankers usually do not cause the berries to drop.



FIG. 2. A. Black-rot blossom blight of a muscadine grape. Several peduncle cankers are on the flower cluster at the left. The two flower clusters on the right are entirely blighted. The leaf at the right is also blighted as the result of severe cankers on the petiole; $\times \frac{1}{2}$. B. Black-rot cankers on the stem of a muscadine grape; $\times 1\frac{1}{2}$.

Black rot does, however, cause a small amount of berry drop on the most susceptible varieties such as Hunt and November in seasons that are

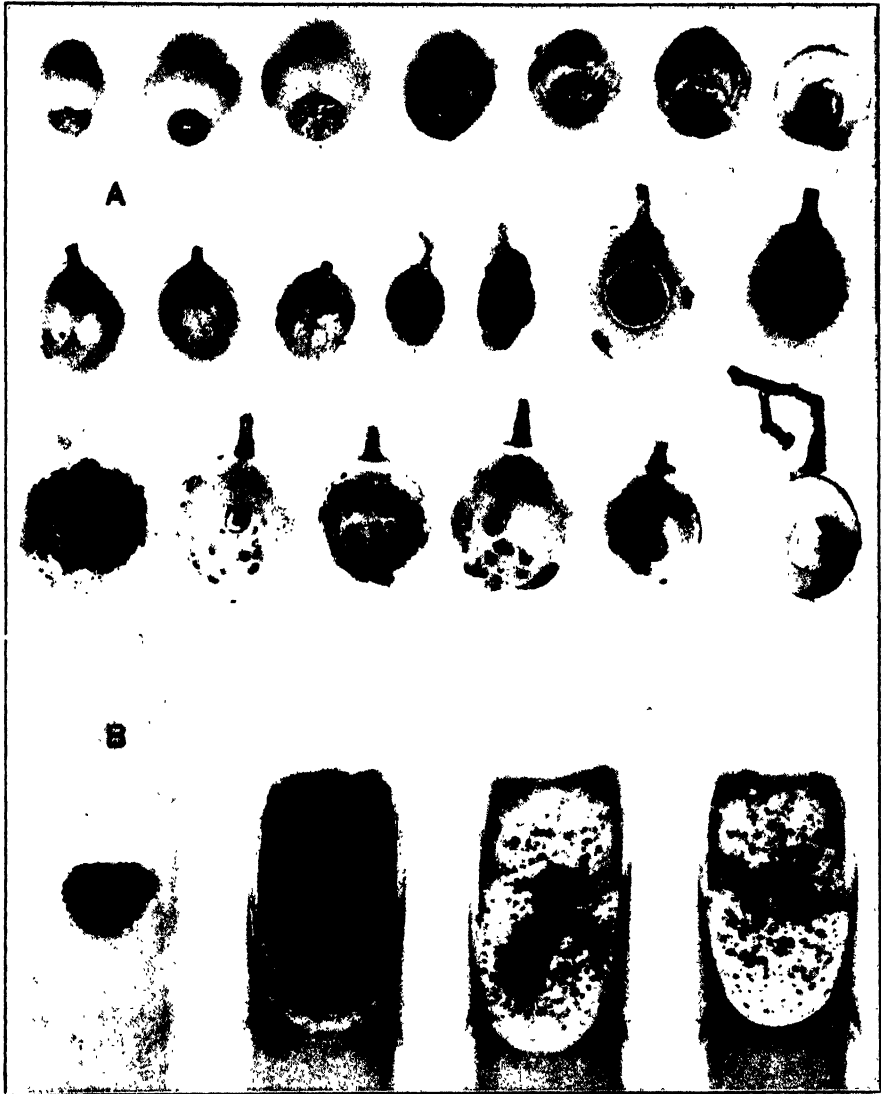


FIG. 3. A. Black-rot scabs and cankers on muscadine berries. Row 1, left to right, a series of berries of the Hunt variety showing the development of a severe canker: the three berries on the right had dropped from the vine; the berry on the extreme right is sectioned to show the deep canker extending inward to the seed. Row 2, berries 1-5, berries of the Hunt variety showing the formation of a shriveled "raisin" resulting from the development of a canker at the point of attachment to the pedicel; these shriveled berries were clinging to the vine; berries 6 and 7, large cankers on berries of the same variety. Row 3, berry 1, a berry of the Dawn variety showing a mosaic-like crust resulting from infection with black rot; berries 2-6, superficial scabs on the surface of Hunt berries; the berry at the extreme right has been sectioned. $\times 1$.

B. *Guignardia bidwellii* in culture tubes on 3 per cent malt agar; all colonies are the same age; left to right, *G. bidwellii* var. *muscadinii* isolated from ascospores from a muscadine grape leaf, *G. bidwellii* isolated from ascospores from berry mummies of a Warren bunch grape, of *Vitis vinifera*, and of an unknown variety of eastern bunch grape. $\times 1$.

especially favorable to the development of the disease. Then large, depressed cankers that extend inward to the seed may form on some berries (Fig. 3, A, row 1). These berries shrivel slightly and fall from the vines. Cankers also may form on the berry close to the point of attachment to the pedicel and interfere with conduction into the berry (Fig. 3, A, row 2, No. 1-5). Such berries shrivel and may either cling to the pedicel or fall to the ground. The shriveled berry appears to be a raisin produced by desiccation rather than a mummy resulting from invasion of the berry by the fungus. The canker does not spread, and the berry becomes reddish or brown rather than black. Any drop of the berries resulting from their infection with black rot occurs when the berries are immature. This disease does not cause a drop of mature berries.

VARIETAL SUSCEPTIBILITY

Observations in a variety vineyard at the Georgia Experiment Station indicated that there was considerable variation in susceptibility to black rot among the muscadine grape varieties represented. These observations were tested by counting infected leaves and berries on 16 named and 8 unnamed varieties. Counts were made just before harvest on five shoots on each of two vines in 1943 and on five shoots on each of four vines in 1944. The number of leaves fallen, the number of remaining leaves infected, the number of spots on each infected leaf, the number of cankers on each stem, the number of berries, and the number of berries infected were recorded for each shoot. Since there was only a small amount of defoliation and black rot was the only disease on the leaves up to harvest time, the number of fallen leaves was included with the number of leaves infected. The best criteria of susceptibility seem to be percentage of berry infection and percentage of leaf infection plus defoliation. Upon the basis of these two criteria the varieties may be separated into three general groups and classified as being very susceptible, of intermediate susceptibility, or resistant.

Upon the basis of berry infection, the varieties November, Howard, and Hunt were very susceptible (24-18 per cent infection); Dawn, Creek, Thomas, Scuppernong, Spalding, and LaSalle were intermediate (11-3.5 per cent infection); and Stuckey, Brownie, Lucida, Big Boy, Irene, Dulcet, and Yuga were resistant (2-0.35 per cent infection). These results on susceptibility of berries were checked in 1945, a season in which diseases were exceptionally severe, by making counts on several of the most susceptible and the most resistant varieties. Fifty berry clusters on each of two vines of each variety were counted, and the percentage of berries infected was recorded. The results were as follows: Hunt, 861 berries—29.27 per cent infected; Howard, 494 berries—16.60 per cent infected; Dawn, 500 berries—14.40 per cent infected; Thomas, 776 berries—1.16 per cent infected; and Dulcet, 825 berries—0.24 per cent infected. The berries of resistant varieties, when they are infected, usually bear only small, superficial scabs; while the berries of susceptible varieties are often severely cankered. This difference in severity of cankers was demonstrated in 1945 by collecting

at weekly intervals from the first of August until after harvest all of the berries that fell to the ground from two vines of each variety and recording the percentage of fallen berries that dropped because of black-rot cankers. Berry drop was severe on all varieties, but little of it was caused by black-rot berry cankers. Cankers caused berry drop on only six varieties, three of which were those classed as very susceptible. The percentages of fallen berries that dropped because of black-rot cankers are as follows: November, 2.34; Hunt, 2.26; Howard, 0.72; Creek, 0.24; Dawn, 0.18; and Spalding, 0.17.

Upon the basis of leaf infection plus defoliation Stuckey, Dawn, Howard, Scuppernong, LaSalle, November, and Hunt were very susceptible (70-60 per cent infection); Brownie, Spalding, Big Boy, Lucida, Yuga, Creek, and Irene were intermediate (59-50 per cent infection); and Dulcet and Thomas were resistant (40-37 per cent infection). While the majority of the varieties are resistant to infection of the berries, most of them are susceptible to infection of the leaves. Furthermore, resistance of the berries is not necessarily correlated with resistance of the foliage. For example, the Stuckey variety, whose berries are resistant, has extremely susceptible foliage.

Upon the basis of both leaf infection and berry infection Dulcet seems to be the most resistant of these 16 varieties. Thomas, Yuga, Irene, and Big Boy also show a considerable degree of resistance. November, Howard, and Hunt appear to be the most susceptible varieties.

IMPORTANCE

Exact data on the effect of black rot on vegetative parts of muscadine grapes could be obtained only by comparing yields of infected vines with yields of vines on which the disease was completely controlled. Control of the disease on vegetative parts has, however, proved too difficult to make possible such a comparison. In the absence of these data only an estimate of the importance of the black-rot disease can be made. Although the percentage of leaves remaining at harvest time on the vines which are infected is high in all varieties, varying from 33 per cent on Thomas to 65 per cent on Stuckey, the amount of defoliation, not all of which can be attributed to black rot, is low. On most varieties it is less than 10 per cent. On Thomas and Dulcet it is approximately 6 per cent, and on Yuga and Creek it is approximately 4 per cent. Exceptions are Stuckey and Scuppernong with 18 and 17 per cent defoliation, respectively. Although a great many leaves are diseased, such leaves generally continue functional. On all varieties the proportion of stems infected averages 63 per cent. Only 2 per cent of the stems are blighted at the tips, however; and axillary buds continue the growth of blighted shoots almost immediately. In view of the fact that muscadine grapes are extremely vigorous, producing an excess of vegetative growth, it seems improbable that either the reduction in functional leaf area as a result of spotting or the small amount of stem tip

blight is of much importance in a properly managed vineyard. Only Stuckey and possibly Scuppernon, on which there is a great amount of leaf infection and a considerable amount of defoliation, appear to be affected by the presence of black rot on the vegetative parts.

Blossom blight appears to be of more importance than leaf infection although here again, since control has not been obtained, no exact data on its influence on yield are available. Blossom blight is occasionally severe on the first flower clusters formed in the spring. There is little blossom blight later in the season. Since muscadine grapes bloom over a long period, it is possible that fruit set late will compensate for that lost as a result of blight of the flower clusters early in the spring. But, even so, severe blossom blight results in irregular ripening of the crop, and this is objectionable because it increases the cost of harvesting.

On most varieties berry infection is negligible. Berry infection must be considered, however, because Hunt, the variety of greatest promise for commercial growers, is one of the most susceptible varieties. Infection of Hunt berries may be as high as 30 per cent, and the cankers may be severe enough to cause the berries to drop. Even on Hunt, however, berry drop resulting from infection with black rot is of little importance. In 1945 thirty-five per cent of the crop on Hunt vines in the Experiment Station variety vineyard dropped to the ground. Bitter rot, the most important single factor, accounted for 55 per cent of this drop. Black-rot cankers on the berries were responsible for only 2 per cent of it. This represents only 0.4 lb. lost because of black rot of a total potential yield of 79 lb. per vine. Since black-rot cankers do not spread in mature tissue, it is improbable that cankers on the pedicels were responsible for any considerable amount of the berry drop. Black rot is, therefore, considered of little importance in reducing yields. This is illustrated by the results from spraying. In 1943 counts of infected berries on Hunt vines showed that 30.7 per cent of the berries were infected on unsprayed vines, while only 14.69 per cent were infected on sprayed vines. This reduction of approximately 50 per cent in amount of infection was not, however, reflected in increased yields. On both the sprayed and the unsprayed vines the average yield was 48 lb. per vine. The belief that black rot is not of primary importance in influencing yield is further substantiated by the fact that, during the past six years in the Experiment Station variety vineyard, the Hunt variety has produced an average annual yield of 66.17 lb. per vine; while Dulcet, the most resistant variety, has produced only 31.17 lb. per vine.

Black rot does, however, affect the quality of the crop. Although the superficial, scabby lesions do not reduce the yield, they damage the appearance of the fruit.

ETIOLOGY

Taxonomy. Observation, first by Dr. B. B. Higgins, of the fact that bunch grape vines growing adjacent to or intertwined with heavily infected

muscadine vines often remain free of black rot suggested that the fungus associated with black rot of muscadine grapes differs in pathogenicity from *Guignardia bidwellii*, the cause of the disease on bunch grapes. Results of inoculation trials have now confirmed this observation. Because this difference in pathogenicity is accompanied by only slight morphological differences, the fungus causing black rot of muscadine grapes is described as a new form of *G. bidwellii*.

***Guignardia bidwellii* f. *muscadinii* f. nov.**

Status perithecialis: Peritheciis nigris, amphigenis, plerumque hypophyllis, punctiformibus, per totum folium dispositis, subinnatis, sphaeroideis, $61.6\text{--}112.0\ \mu$ (medio. $84.28\ \mu$ diam., poro praeditis; ascis fasciculatis, aparaphysatis, cylindraceis vel clavatis, $36.4\text{--}56.0 \times 11.76\text{--}16.8\ \mu$ (medio $43.76 \times 14.28\ \mu$), sessilis vel breve stipitatis, bitunicatis, octosporis; ascosporis hyalinis, continuis, obovatis vel oblongis, rectis vel plerumque leniter inaequalibus, $13.5\text{--}17.25 \times 6.0\text{--}9.0\ \mu$ (medio, $14.92 \times 7.24\ \mu$), biseriatis vel inordinatis.

Hab. in verno in foliis dejectis *Vitis rotundifoliae* Michx., Experiment, Georgia.

Status spermogonicus: Spermogoniis nigris, punctiformibus, amphigenis, plerumque hypophyllis, cum stromatibus carpogonialibus per totum folium dispersis, subinnatis, globosis, $44.8\text{--}78.4\ \mu$ diam., poro praeditis; spermatis hyalinis, bacillaribus, plerumque $2.5 \times 1\ \mu$, intus loculo centrali efformantis.

Hab. in autumno in foliis dejectis *Vitis rotundifoliae*.

Status conidicus: Pyrenidiis nigris, punctiformibus, amphigenis in foliis in maculis rufo-brunneis, orbicularibus, 1–12 mm. lat. vel in ramis et fructibus in laesionibus nigris effortmantis, globosis, $58.8\text{--}126.0\ \mu$ diam., poro praeditis; pycnosporis hyalinis, non-septatis, obovatis, oblongis, vel subglobosis, utrinque obtusculis, $7.98\text{--}13.30 \times 5.32\text{--}7.5\ \mu$ (medio $10.16 \times 6.40\ \mu$), ex hyphis brevis intus loculo centrali oriundis.

Hab. in verno, aestivo, atque autumno in laesionibus in foliis, ramis, et fructibus vivis *Vitis rotundifoliae*.

Collections of type material have been deposited in the Farlow Herbarium, Harvard University, in the Mycological collections of the Bureau of Plant Industry, U. S. Department of Agriculture, and in the herbarium of the Royal Botanic Garden at Kew, Surrey, England.

The form *muscadinii* differs from *Guignardia bidwellii* in pathogenicity, in appearance and rate of growth of colonies in culture, in the smaller size of perithecia, and in the slightly larger size of ascospores and pycnospores. These differences will be brought out in detail in the following account. They are not considered sufficient basis for the erection of a new species or variety.

Morphology. Only the pyrenidial stage of *Guignardia bidwellii* f. *muscadinii*, which belongs in the form genus *Phoma*, is associated with black-

rot lesions on muscadine vines during the growing season. Primary infection of the leaves and stems as soon as they emerge from the buds in the spring is accomplished by ascospores from perithecia on overwintered leaves and by pycnosporos from cankers on stems infected during the previous season. The spores are carried by air currents or raindrops to the surface of the new growth where, under suitable moisture conditions, they germinate. Although entrance of the germ tubes has not been observed directly, evidence obtained from inoculations indicates that infection takes place through the upper surface of the leaf as readily as through the lower surface. Since the upper epidermis of the leaf is not provided with stomata, the germ tubes must be capable of penetrating the epidermis directly. Within the leaf the mycelium spreads intracellularly through the epidermis and mesophyll without producing externally visible symptoms for about three weeks. At the end of the incubation period the invaded cells die, producing the characteristic black-rot lesions. A similar invasion of immature tissue of petioles, stems, tendrils, flower clusters, and berries results in the production of cankers. The cells of the mycelium filling the dead epidermal cells become thick-walled and dark-colored, producing a black crust over the surface of the canker. On berries the infected tissue is separated from the healthy tissue beneath by layers of cork cells.

Pyrenidia develop rapidly within the lesions as the infected tissue dies. The pyrenidium originates as a spherical mass of pseudoparenchymatous tissue beneath the epidermis. Within the pyrenidium a central cavity is formed, and cells lining the cavity produce short conidiophores which abstrict pycnosporos from their tips. The apex is provided with a pore through which the pycnosporos which now fill the cavity are liberated. The pycnosporos are sub-globose, obovate, or oblong, hyaline, and nonseptate. They immediately initiate secondary infections.

The fungus survives the winter on infected stems and leaves. No spores of *Guignardia biduellii* f. *muscadinii* have been found on overwintered berries. This may be because the berries, as soon as they fall to the ground, are invaded by various fungi that are more vigorous saprophytes. Functional pyrenidia survive the winter in cankers on the stems, but these cankers are excised by growth of the stems during the second season. Pycnosporos have also been found in abundance on infected stems which were pruned off in the fall and left lying on the ground over winter.

Pyrenidia on leaves do not survive the winter, but perithecia are produced abundantly on over-wintered leaves. After the leaves die and fall from the vines at the end of the growing season, the fungus spreads throughout the leaf. From October to December, spermogonia and perithecial initials develop in the dead leaves. They are formed over the entire surface of the leaf outside of the original lesions. Externally spermogonia are similar to pyrenidia except for their smaller size. The spermogonium is composed of a pseudoparenchymatous wall surrounding a central cavity filled with hyaline, unicellular, bacilliform spermatia. The spermatia are liberated through a pore in the apex of the spermogonium.

Perithecial initials develop slightly later than spermiogonia but are externally indistinguishable from them. They differ internally, however, in that no central cavity is formed. The perithecial initial is filled with a mass of large, thin-walled, hyaline pseudoparenchymatous cells. Several deeply-staining carpogonial cells lie in the pseudoparenchyma within each perithecial initial. From these cells a fascicle of asci is produced in the base of the developing perithecium during January. As the asci develop the pseudoparenchyma is crushed and disintegrated leaving a central cavity occupied by the asci. The ascospores mature during late March. The mature perithecium consists of a spherical, dark-colored, pseudoparenchymatous wall surrounding a central cavity containing a fascicle of paraphysate asci. At the apex the wall is provided with a pore through which the ascospores are discharged. Mature asci are sessile or short-stipitate and are cylindrical to clavate. Each contains eight biserially arranged ascospores. The ascospores are obovate to oblong, hyaline, and non-septate. When the asci are moistened, they discharge their ascospores forcibly one after another. Ascospore discharge continues for four or five weeks during April and May.

Guignardia bidwellii and its form *muscadinii* differ in symptoms produced on the host plants as well as in morphology. On bunch grapes infection with *G. bidwellii* results in the complete mummification of the berries, while on muscadine grapes *G. bidwellii* f. *muscadinii* causes only a superficial scab or canker of the berries. It is quite possible, however, that this difference in symptoms is the result of host reaction. The differences in morphology also are slight (Table 1). Perithecia of *G. bidwellii* were larger than those of the form *muscadinii*, the difference in average diameter being 62.2 μ . Since perithecia of *G. bidwellii* are produced only on berries and those of the form *muscadinii* only on leaves, the larger size of the perithecia of *G. bidwellii* may be a result of growth on a richer substrate. This will not, however, explain the difference in size of ascospores since the ascospores of the variety were 1.4 μ longer and 0.6 μ wider than those of the species. There was little difference in size of pycnidia. The pycnosporos of the form were slightly longer than those of *G. bidwellii*, the difference being 1.0 μ in average length. A similar difference was apparent in the size of pycnosporos produced on leaves of *Vitis vinifera* inoculated with the two fungi. On the same host the difference in average length of pycnosporos was 0.8 μ .

Growth in Culture. *Guignardia bidwellii* f. *muscadinii* was obtained in culture from ascospores by suspending pieces of over-wintered muscadine leaves bearing mature perithecia from the top of a Petri dish so that the ascospores were discharged upon the agar below. Cultures were obtained also from infected tissue of muscadine stems, leaves, and berries by planting bits of surface-sterilized tissue cut from the margins of black-rot lesions on tapwater agar. Isolates from all sources produced similar colonies on 3 per cent malt agar. The colonies (Fig. 3, B) were slow-growing,

TABLE 1.—A comparison of the morphology of *Guignardia bidwellii* and its form *muscadinii*

Part	<i>G. bidwellii</i>		<i>G. bidwellii</i> form <i>muscadinii</i>	
	On bunch grape		On muscadine grape	
Perithecia	95.2-198.8 μ	(av. 50 = 146.5 μ)	61.2-112.0 μ	(av. 50 = 84.3 μ)
Ascospores	11.3-16.5 \times 6.0-6.8 μ	(av. 100 = 13.5 \times 6.6 μ)	13.5-17.3 \times 6.0-9.0 μ	(av. 100 = 14.9 \times 7.2 μ)
Pycnidia				
(on leaf)	61.6-95.2 μ	(av. 25 = 79.4 μ)	59.8-106.4 μ	(av. 25 = 82.9 μ)
(on berry)	114.8-196.0 μ	(av. 25 = 145.4 μ)	70.0-126.0 μ	(av. 25 = 97.9 μ)
(on stem)	98.0-137.2 μ	(av. 25 = 113.2 μ)	61.6-117.6 μ	(av. 25 = 91.4 μ)
Pycnospores	7.5-11.5 \times 5.6-8.0 μ	(av. 100 = 9.2 \times 6.7 μ)	8.0-13.3 \times 5.3-7.5 μ	(av. 100 = 10.2 \times 6.2 μ)
	On vinifera grape		On vinifera grape	
Pycnidia	68.0-149.6 μ	(av. 25 = 113.2 μ)	68.0-149.6 μ	(av. 25 = 97.5 μ)
Pycnospores	8.0-12.0 \times 6.4-6.7 μ	(av. 25 = 8.5 \times 6.6 μ)	8.0-13.3 \times 6.0-6.7 μ	(av. 25 = 9.3 \times 6.3 μ)

black, hemispherical masses of plectenchymatous stromatic tissue densely covered with pseudoparenchymatous pycnidia. Pycnosporos, produced abundantly in these cultures after three to four weeks, were identical with those found in black-rot lesions on muscadine vines. Pycnospore production ceased after several weeks, and no pycnosporos were formed in transfers of the original cultures. In transfers a scanty white mycelium developed over the colonies, partially covering the black stromatic mass and extending slightly beyond its margin.

For comparison, *Guignardia bidwellii* was isolated from ascospores from perithecia on over-wintered black-rot berry mummies from the following three different bunch grapes: (1) a European grape near Conyers, Georgia, (2) an undetermined variety of native grape at the same locality, and (3) a Warren grape near Experiment, Georgia. The isolates from all three sources were similar to one another but different from those of the form *muscadinii*. On 3 per cent malt agar the three isolates produced more rapidly growing colonies of white mycelium which soon became gray or black at the surface of the medium (Fig. 3, B). Pycnidia were scattered separately in the mycelium. The pycnosporos formed in these cultures were similar to those formed by *G. bidwellii* f. *muscadinii*. Pycnosporos were again produced in the first transfers from the original cultures, but they were not formed in subsequent transfers.

In addition to the differences in type of colony formed by *Guignardia bidwellii* and its form *muscadinii* and in rates of growth in culture there was a slight difference in size of pycnosporos produced by the two fungi under identical conditions on the same culture medium. The pycnosporos of *G. bidwellii* were $7.5\text{--}12.0 \times 6.0\text{--}9.0 \mu$ (av. 125, $9.17 \times 6.97 \mu$); those of the form *muscadinii* were $7.80\text{--}14.63 \times 5.32\text{--}9.31 \mu$ (av. 125, $10.09 \times 6.92 \mu$). Although there was no difference in width, the pycnosporos of *G. bidwellii* f. *muscadinii* exceeded those of *G. bidwellii* both in maximum length and in average length. The difference in maximum length was 2.63μ , while the difference in average length was 0.92μ .

Inoculations. Inoculation experiments were designed to determine (1) the pathogenicity of *Guignardia bidwellii* and *G. bidwellii* f. *muscadinii* on several species and varieties of *Vitis*, (2) the differences in pathogenicity between the species and its form, and (3) the genetic connection of the perfect and the imperfect stages of both fungi. All inoculations were made in the greenhouse where there was no natural infection. The inoculum used was suspensions of pycnosporos obtained by macerating pycnidia from cultures or from host lesions in sterile tap water. Host material was furnished by small vines grown in 6- or 8-inch pots. In making inoculations single, vigorously growing shoots bearing leaves in various stages of development were selected. The number of leaves on each shoot and the size of each leaf were recorded. Drops of inoculum were applied along the stem and to each leaf on the shoot. After permitting the drops of inoculum to dry, the inoculated plants were placed in a moist chamber for 48 hours.

The plants were then returned to benches in the greenhouse and kept under observation for at least one month. Control plants included in each experiment were treated similarly except for the fact that they were not inoculated.

Only the muscadine grape was inoculated with pycnosporos from field material of *Guignardia bidwellii* and *G. bidwellii* f. *muscadinii* in July, 1943. Six shoots bearing a total of 70 leaves were inoculated with pycnosporos of *G. bidwellii* f. *muscadinii* obtained from leaf spots on muscadine vines. All six shoots became infected. Leaf spots or petiole cankers or both appeared on 31 of the 70 leaves. Five of the leaves were killed. The stems of two of the shoots were cankered, and their tips were blighted. Eleven control shoots with 85 leaves remained free of infection. Eight shoots with 64 leaves were inoculated with pycnosporos of *G. bidwellii* from leaf spots on a Warren bunch grape, and six shoots with 49 leaves were inoculated with pycnosporos from leaf spots on an undetermined species of wild bunch grape. None became infected. In these experiments *G. bidwellii* f. *muscadinii* produced 100 per cent infection on muscadine shoots; while *G. bidwellii* produced no infection on similar shoots.

During June in 1944 and 1945 more extensive inoculations with both *Guignardia bidwellii* and *G. bidwellii* f. *muscadinii* were made on four different hosts. (1) a European bunch grape (*Vitis vinifera* Linn.), (2) a muscadine grape (*V. rotundifolia*), (3) a Warren bunch grape (*V. bourquina* Munson ex Viala), and (4) a Niagara bunch grape (*V. labrusca* Linn.). Inoculum of *G. bidwellii* f. *muscadinii* was a suspension of pycnosporos produced in cultures of ascospores from over-wintered muscadine leaves. Inoculum of *G. bidwellii* was suspensions of pycnosporos produced in cultures of ascospores from over-wintered berries of three different bunch grapes; a European, a Warren, and an undetermined variety of native grape. Since inoculum of *G. bidwellii* from these three different sources produced similar results, they will not be considered separately. The results of these inoculations are in table 2. During these experiments, the control shoots remained free of infection. *G. bidwellii* f. *muscadinii* produced 100 per cent infection of muscadine shoots but did not infect Warren or Niagara shoots. On the other hand, while *G. bidwellii* infected almost 90 per cent of the Warren and Niagara shoots, it did not infect muscadine shoots. Both, however, produced 100 per cent infection of vinifera shoots. The two fungi were reisolated from lesions on the inoculated shoots by planting bits of surface-sterilized infected tissue on tap-water agar. Both *G. bidwellii* and the form *muscadinii* were reisolated unchanged from the vinifera grape.

The incubation period of *Guignardia bidwellii* on all bunch grapes was approximately two weeks. The incubation period of *G. bidwellii* f. *muscadinii* on muscadine grapes was approximately three weeks. On the vinifera grape, however, its incubation period was two weeks, the same as that of *G. bidwellii*. The longer incubation period of the form *muscadinii* on muscadine grapes appears, therefore, to be the result of host reaction, and it does not constitute a physiological difference between *G. bidwellii* and its variety.

The low percentage of leaf infection (Table 2) may be explained by the fact that only immature host tissue is susceptible. If only immature leaves were selected for inoculation, the percentage of leaves infected might approach 100 per cent. For this reason the differences in amount of leaf infection are not considered significant. They are simply a result of variation in the shoots inoculated, young rapidly growing shoots presenting a greater proportion of immature susceptible leaves than older shoots.

On muscadine vines spots appeared only on the first to the seventh leaf back of the growing tip. These leaves at the time of inoculation varied from tiny leaves not yet expanded to leaves up to 4.7 cm. across. No spots ever appeared on larger, more nearly mature leaves farther back from the tip of the shoot. Apparently the fungus spread within the leaf tissue without

TABLE 2.—*Results of inoculation trials with Guignardia bidwellii on grapes*

Hosts	<i>G. bidwellii</i>					<i>G. bidwellii</i> f. <i>muscadinii</i>				
	Shoots inoculated	Shoots infected	Leaves inoculated	Leaves infected	Stems infected	Shoots inoculated	Shoots infected	Leaves inoculated	Leaves infected	Stems infected
	Num-ber	Per-cent	Num-ber	Per-cent	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Per-cent
<i>Vitis vinifera</i>										
Inoculated	7	100	45	73.3	57.1	5	100	62	43.6	40
Control	2	0	13	0	0	3	0	25	0	0
<i>Vitis labrusca</i>										
Inoculated	10	100	40	47.5	20.0	5	0	28	0	0
Control	6	0	28	0	0	3	0	17	0	0
<i>Vitis bourquina</i>										
Inoculated	11	72.7	58	25.9	36.3	5	0	35	0	0
Control	4	0	31	0	0	2	0	18	0	0
<i>Vitis rotundifolia</i>										
Inoculated	11	0	112	0	0	8	100	80	26.3	100
Control	7	0	66	0	0	8	0	61	0	0

producing any external symptoms so long as the tissue was immature. As the leaf tissue approached maturity the extension of the fungus ceased, and the invaded tissue died and appeared as a spot in the leaf. The spots, therefore, had attained almost their full dimensions when they first appeared and did not enlarge in the mature leaves. For this reason the spots on leaves that were young when inoculated were large. In leaves that were almost mature when inoculated the fungus spread little, and, consequently, the spots were small. Since, however, the older leaves presented a greater surface area to the inoculum, the spots on these leaves were numerous. Apparently petiole tissue remains susceptible longer than leaf-blade tissue. Petiole cankers appeared on the first to the eighth leaf back of the growing tip of the shoot and on leaves as much as six cm. across at the time of inoculation. Severe petiole cankers sometimes resulted in the death of the entire leaf. Stem tissue was infected only from the first to the fifth nodes back of the growing tip. Occasionally the entire stem tip was blighted.

These results demonstrate that both *Guignardia bidwellii* and *G. bidwellii* f. *muscadinii* are capable of infecting leaves and stems of grapes and of producing typical black-rot lesions on them and that only immature host tissue is susceptible to infection by either organism. They show further that there is a difference in pathogenicity between *G. bidwellii* and its form. Of our native grapes *G. bidwellii* attacks only bunch grapes, while the form *muscadinii* attacks only muscadine grapes. Both fungi, however, are pathogenic to the European grape, producing similar symptoms on the leaves and stems. The fact that ascospores from perithecia on over-wintered leaves or berries produce cultures in which the pycnidial stage is formed and that pycnosporos from these ascospore cultures produce black-rot lesions bearing typical pycnidia when inoculated on the leaves and stems of the host demonstrates the genetic connection of the perfect and imperfect stages of each of the two fungi.

CONTROL

In order to determine the protection against black rot afforded by spraying, experiments were conducted in an acre vineyard of the Hunt variety at the Georgia Experiment Station during the seasons 1942-1944. Half of the vineyard was not sprayed, while the other half was given four sprays each season. A 4-4-50 Bordeaux mixture was applied as follows: two sprays prior to blooming, one after the fruit was set, and the last when the fruit was half grown. Since there was some injury to young foliage, a 2-3-50 Bordeaux mixture was substituted for the first spray.

Spraying resulted in no increase in yields. The average yields per vine in the sprayed and in the unsprayed halves of the vineyard were as follows: 1942, sprayed—51 lb., unsprayed—69 lb.; 1943, sprayed—48 lb., unsprayed—48 lb.; 1944, sprayed—80 lb., unsprayed—84 lb. The average annual yields per vine for the three-year period were: sprayed—59.7 lb. (average of same vines for three years prior to test, 61 lb.), unsprayed—67 lb. (average of same vines for three years prior to test, 66 lb.).

In order to determine whether spraying reduced the amount of infection with black rot, even though it did not result in increased yields, counts were made in the vineyard immediately before harvest in 1943. Counts were made on five shoots on each of 20 vines, that is, on a total of 100 shoots in both the sprayed and the unsprayed halves of the vineyard. The results are given in table 3. The application of four sprays was not very effective in reducing the amount of black rot on the vegetative parts. It produced only a slight reduction in the percentage of leaves infected and in the percentage of stems cankered. More frequent spraying would probably result in a greater reduction. It is difficult to keep the foliage covered with spray, however, because of the rapid growth of the shoots. For example, the rate of growth of the Hunt variety was determined by tagging 25 shoots scattered throughout an acre vineyard and measuring the elongation of the stems and counting the number of new leaves formed at one- or two-week intervals during the 18 weeks from April 11 to August 16, 1945. The weekly rate of

growth was fairly uniform throughout this period except for the three weeks from April 19 to May 9 when unseasonable cold weather retarded growth. During the 18 weeks, the stems elongated an average of 2.98 in. per week and added an average of 2.07 new leaves per week. The most vigorous shoot averaged 6.88 in. stem elongation and produced an average of 3.78 new leaves per week. The highest rate of growth recorded for any shoot in a single week was a stem elongation of 10 in. with the addition of six new leaves. It is obvious that, even if the vines were sprayed at weekly intervals, a great amount of the most susceptible tissue would be exposed to infection shortly after each spray was applied. Apparently it would be impossible to obtain complete control of black rot by protective spraying in the presence of a continuous source of inoculum such as is furnished by unsprayed check rows.

TABLE 3.—*Effect of spraying on amount of black-rot infection on Hunt muscadine grapes at the Georgia Experiment Station in 1943*

Data recorded	Vines not sprayed	Vines sprayed	Reduction from spraying <i>Per cent</i>
No. of shoots	100	100	
No. of leaves	2271	2159	
Percentage of leaves infected and lost	52.58	49.33	6.18
Av. no. of spots per infected leaf	11.03	8.25	25.20
Percentage of stems cankered	74.00	66.00	10.81
Av. no. of cankers per infected stem	6.73	5.55	17.53
No. of fruit clusters	165	153	
Percentage of fruit clusters with pedicels infected	18.18	13.14	27.72
Av. no. of cankers per infected cluster	1.40	1.25	10.71
Number of berries	1300	1168	
Percentage of berries infected	30.70	16.01	47.85
Av. no. of cankers per infected berry	2.84	1.96	30.99

Of course, spraying an entire isolated vineyard in conjunction with the elimination of neighboring wild vines and cultivated vines which cannot be properly cared for, thorough pruning, and the disposal of all litter by burning during the winter would probably greatly reduce the amount of infection or even eliminate black rot in the vineyard.

Although only two of the four sprays were applied to the berries, these two sprays reduced the number of berries infected by approximately 50 per cent and also reduced the number of scabs and cankers on the infected berries by 30 per cent. It seems certain that more frequent spraying of the berries, perhaps at two-week intervals from the time the berries are set until they are full size, would give them adequate protection. Spraying the berries is difficult, however, because they are covered by a dense growth of stems and leaves.

DISCUSSION

Pathogenicity. The results obtained in the inoculation trials reported here are of interest because of the disparity in the results obtained by previous workers. Prior to 1900 several French pathologists reported studies in

which they apparently were able to demonstrate the pathogenicity of *Guignardia bidwellii* on bunch grapes as well as the genetic connection of the perfect and the imperfect stages of the fungus. Viala and Ravaz (10) inoculated berries with pycnosporos and produced typical black-rot lesions on which pycnosporos and later spermogonia formed. They also obtained infection of leaves inoculated with ascospores from overwintered berries, proving the pathogenicity of *G. bidwellii*, the genetic connection of the pycnidial and perithecial stages of the fungus, and the identity of the fungus on the leaves with that on the berries. Prunet (5) obtained infection of leaves by inoculating them with pycnosporos. He observed the process of infection microscopically and found that the germ tubes penetrate the cuticle directly. He discovered that the incubation period depends upon weather conditions and that it varies from 22-25 days in April to 10-12 days in July. He also found that only young, growing leaves are susceptible. Mature leaves were not infected. Leaves that were not quite mature when inoculated but matured during the incubation period also were not infected. The germ tubes penetrated the cuticle of these leaves, but growth of the fungus within the host was arrested. The spots usually presented their full dimensions when first evident. Soursac (9) later attempted by means of inoculation trials to determine the relative resistance of various species of *Vitis*. He was able to obtain infection in the greenhouse only on *V. vinifera*, but his field inoculations were successful on a number of species of *Vitis*. Although these results seem to be conclusive, Reddick (6) reported that in experiments in New York he was unable to obtain proof of the pathogenicity of *G. bidwellii* on bunch grapes although he made thousands of inoculations both indoors and in the field. The results of my inoculation trials prove again the pathogenicity and connection of stages of *G. bidwellii* on bunch grapes and are in complete accord with those of Viala and Ravaz, Prunet, and Soursac. They demonstrate further the pathogenicity and genetic connection of the perfect and imperfect stages of *G. bidwellii* f. *muscadinii* on muscadine grapes. It is difficult to explain Reddick's failure to obtain infection with *G. bidwellii* unless his inoculations were made on mature tissue.

Forms of Guignardia bidwellii. The fact that the fungus associated with black rot of muscadine grapes differs in pathogenicity from *Guignardia bidwellii*, cause of black rot of bunch grapes, is of practical importance for the following reason. Since *G. bidwellii* f. *muscadinii* does not infect bunch grapes, it should be possible to plant native bunch grapes in the vicinity of muscadine grapes without danger of the disease spreading from the muscadine grapes to the bunch grapes. It should, therefore, be unnecessary to make costly attempts to control black rot on the muscadine grapes in order to remove a source of inoculum for the infection of the bunch grapes. On the other hand, since *G. bidwellii* f. *muscadinii* will readily infect *Vitis vinifera*, diseased muscadine vines would be a constant threat to plantings of European grapes in their vicinity.

The existence of a distinct form of *Guignardia bidwellii* explains Soursac's failure to obtain infection on *Vitis rotundifolia*. Soursac (9), using

inoculum of *G. bidwellii* from a single source, obtained infection on the following species which are listed in order of increasing resistance: *Vitis vinifera*, *V. arizonica* Engelm., *V. californica* Benth., *V. labrusca*, *V. rubra* Michx., *V. monticola* Buckley, *V. coriacea* Shuttleworth, and *V. rupestris* Schiele. In another experiment he obtained infection also on *V. Lincecumii* Buckley, and *V. aestivalis* Michx. His inoculations produced no infection on *V. rotundifolia* because, as is now evident, *V. rotundifolia* is susceptible only to *G. bidwellii* f. *muscadinii*.

It seems probable that there are other forms of *Guignardia bidwellii* in addition to the form *muscadinii*. Soursac (9) failed to obtain infection on four American species of bunch grape, *Vitis riparia* Michx., *V. cordifolia* Lam., *V. cinerea* Engelm., and *V. candicans* Engelm., although black rot has been reported on all four of these species (8). This suggests the possibility that there are undescribed forms of *G. bidwellii* which attack only certain species of bunch grapes. Furthermore, black rot has been reported on Virginia Creeper (*Parthenocissus quinquefolia* (L.) Planch.) (1, p. 113R) and on Boston ivy (*P. tricuspidata* (Sieb. and Zucc.) Planch.) (2) and has been found on both of these species in Georgia. Preliminary inoculation trials indicate that the fungus associated with the disease on these two species, while it is morphologically similar to *G. bidwellii*, differs in pathogenicity from both *G. bidwellii* and *G. bidwellii* f. *muscadinii*. Further inoculation experiments will probably demonstrate that this is a distinct form of *G. bidwellii* which attacks only *Parthenocissus* species.

Importance of Black Rot on Muscadine Grapes. Although black rot is a common and conspicuous disease, it seems to have little direct effect upon yield of muscadine grapes. It is difficult to control; and control measures beyond good cultural practices (4, 7) such as selection of a suitable vineyard site, proper cultivation, fertilization, pruning, and vineyard sanitation would hardly be profitable. Upon the basis of present evidence spraying cannot be recommended for the control of black rot. With proper management good crops may be produced in the presence of this disease without any special measures being directed toward its control.

The only appreciable damage resulting from black rot is the lowering of the quality of the crop because of the presence of scabs on the berries. This is true of only a few varieties among which, however, is the most important commercial variety, the Hunt. This lowering of quality is of little importance at present because the crop is usually sold in bulk to wineries, and the dry, superficial scabs do not interfere with the use of diseased berries for the extraction of juice. If the crop should become important as a fresh or frozen fruit for table use, black-rot scabs would be objectionable.

Although there are varieties of good quality with resistant foliage and with berries that are almost immune, such as Dulcet and Thomas, these varieties do not yield as well as Hunt, a relatively susceptible variety. At present resistance to black rot is not, therefore, of primary importance in the selection of a variety. If, as seems improbable, black rot should become of major importance, it is well to know that there are resistant varieties of good

quality available. Furthermore, since resistance is present in some varieties, it should be possible to combine resistance with high yield and quality in future breeding work.

SUMMARY

Black rot, a common and conspicuous disease of muscadine grapes (*Vitis rotundifolia* Michx.), is primarily a disease of the vegetative parts. It causes a spot of the leaves, a canker or blight of the petioles, tendrils, stems, and flower clusters, and a superficial scab or canker of the berries. It causes little defoliation and only a negligible amount of berry drop. Black rot has little effect on yield and appears to be of minor importance.

Muscadine varieties vary considerably in degree of susceptibility to black rot. Some varieties, such as Dulcet and Thomas, have relatively resistant foliage and berries which are almost immune. These varieties, however, produce lower yields than Hunt, one of the most susceptible varieties.

The fungus associated with black rot on muscadine grapes differs in pathogenicity from *Guignardia bidwellii* (Ell.) Viala and Ravaz, cause of black rot of bunch grapes. Only slight morphological differences both in culture and in the field are associated with this difference in pathogenicity. The fungus is described, therefore, as a new form, *G. bidwellii* f. *muscadinii*. Inoculation trials have shown that of our native grapes *G. bidwellii* attacks only bunch grapes, while the form *muscadinii* attacks only muscadine grapes. Both, however, are capable of infecting the European grape, *Vitis vinifera*. Inoculations have also demonstrated the genetic connection of the pycnidial and perithecial stages of each of these fungi.

Experiments have produced no evidence that black rot on muscadine grapes can be profitably controlled by spraying. Control measures beyond good cultural practices necessary for the maintenance of vigorous growth are not recommended.

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STUDIES ON THE CAUSE OF STEM-END BROWNING IN GREEN MOUNTAIN POTATOES

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The term stem-end browning has been applied to a distinct type of internal discoloration in tubers of the Green Mountain (Fig. 1), Irish Cobbler, and a few other less common potato varieties. It is characterized by a marked necrosis of the xylem, phloem, and adjacent parenchyma cells of the stem end of the tuber (7). It rarely extends more than one-half inch beyond the stem scar. Very few diseased tubers are found at digging time but in some lots a high percentage of the tubers may develop the abnormality in storage. Maximum development is obtained by storing for about 100 days at 50° F. (4, 5, 6, 11).



FIG. 1. Stem end browning in Green Mountain tubers. (Photograph by M. T. Hilborn.)

Folsom and Rich (7) could find no evidence that the disease was caused by a virus. It has recently been shown, however, that in the case of Green Mountains the amount of stem-end browning in a crop is definitely related to the source of seed or, more specifically, to the commercial strain used (9). It was further demonstrated that in this variety there are two types of strains, differing in the tendency of tubers to develop stem-end browning. One type consistently produces a low percentage of tubers that develop the disease while two to seven times as many tubers of the other type may be diseased when grown and stored under identical conditions. Within a given strain, all tuber lines are equally predisposed to the disease. It is the purpose of this report to present data indicating that this difference in behavior

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among strains is apparently due to the presence of a virus, or virus strain, in those potato strains that normally produce a crop with a high incidence of stem-end browning.

The words "susceptible" and "resistant" as used herein are relative terms and are used to describe differences in tendency or predisposition to develop tuber symptoms referred to as stem-end browning. The term "Green Mountain strain" is used to differentiate between different commercial strains or growers' stocks. Although some of these are known by specific names, it does not necessarily follow that each differs genetically from the others.

EXPERIMENTAL WORK

The Green Mountain variety was used in all experiments. All field experiments were on Aroostook Farm, Presque Isle, Maine, or on an isolated seed farm in northern Maine. All tubers were stored at 50° F. for 100 days or longer before they were examined for stem-end browning.² The objective method described by Folsom and Rich (7) was used to differentiate stem-end browning from net necrosis.

Serological Tests. During the early stages of the work, serological technique as described by Chester (3) was used in an attempt to detect the presence of a virus in tubers with stem-end browning. Some of the anti-sera produced would give positive precipitin tests with most but not all stem-end browning tubers and negative tests with most but not all normal tubers. The results were often erratic, and at the time no explanation could be given for the samples with anomalous behavior, consequently the work was discontinued. At the time, the existence of resistant and susceptible strains was not realized. Following the discovery of the different behavior of strains, the earlier data were re-analyzed. It became clear that when the juice of what appeared to be a susceptible strain had been used for intraperitoneal injection into rabbits and the juice of an apparently resistant strain for absorption of the serum, the anti-serum thus produced gave positive precipitin reactions with potatoes selected from apparently susceptible strains and negative tests with those from strains that appeared to be resistant. In the latter case it did not matter whether or not the tuber had developed stem-end browning. In the former case, both normal and diseased tubers gave a positive reaction but the titer was much higher in the case of those showing stem-end browning. The data were interpreted therefore as indicating either the presence of a virus in the susceptible strains but not in the resistant ones or the presence of different strains of the same virus in the two types of Green Mountain potato.

Cage Experiments. As most potato viruses are spread by insects, a test was made of the effect of exclusion of insects on the incidence of stem-end browning. A field cage was constructed of aster cloth, half of which was entirely enclosed, and the other half left open at the sides to simulate the conditions inside the cage yet allow entry of insects. Both areas, as well as

² Acknowledgment is made to Michael Goven for making the examinations.

an adjoining unshaded and uncaged area, were planted with a resistant and a susceptible strain in alternate rows. To doubly insure the exclusion of insects, the caged plants were sprayed at intervals with nicotine sulphate. Half of the shaded rows and half of the unshaded rows were likewise sprayed with nicotine. The aphid population of the uncaged and unsprayed rows was rather high. The data in table 1 show that the tubers from the caged rows developed approximately the same amount of stem-end browning as did those from the others.

TABLE 1.—*Stem-end browning in Green Mountain tubers produced under a field cage*

Where grown in 1943	Insecticide spray, 1943	Stem-end browning			
		1943 ^a		1944 (field planting) ^b	
		Minne- sota lot	Keswick strain	Minne- sota lot	Keswick strain
				Fields 1 and 2 ^c	Field 2 ^d
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Cage	Nicotine sulphate	4.5	34.4	9.3	40.3
Shaded area	Nicotine sulphate	6.2	31.3
	No spray	4.5	38.6
Open area	Nicotine sulphate	6.3	25.1
	No spray	4.7	30.1	13.0	39.3

^a Figures based on 500 to 700 tubers.

^b The 1943 crop was replanted in 1944 in an open field. Those grown outside the cage in 1943 were tuber indexed before planting. Tubers with leafroll, mosaic, etc., were discarded.

^c Averages for 700 or more tubers from each field. Five single rows, each 29 ft. long, in Latin Square design, were used in Field 1; numerous small samples planted at random were used in Field 2. The figures for Field 2 were 9.5 per cent for the progeny of the cage-grown material and 13.8 per cent for the other sample.

^d See footnote ^c for size of sample.

Samples were replanted the next year. In the case of the susceptible strain, the progeny of the cage-grown samples developed the same amount of stem-end browning as did that of the field-grown samples. In the case of the other lot, however, the progeny of the cage-grown samples produced less stem-end browning than did that of field-grown seed. The difference was not large and may not have been significant. The possible significance of the apparent increase will be discussed later. For the present the important thing to consider is that tubers of the resistant strain still developed much less stem-end browning than did those of the susceptible strain.

The Effect of Continued Propagation of Resistant Strains in Maine. The Green Mountain strain first recognized as being fairly resistant to stem-end browning was obtained from Minnesota. Consequently an experiment designed to test the effect of continued propagation of this strain was initiated. In 1942, this strain was obtained from a grower in Minnesota and propagated the following years either on a seed plot on Aroostook Farm or on an

isolated seed farm in northern Maine. These seed plots were rogued and harvested early to prevent the spread of leafroll and mosaic. Other lots of the strain were planted in ordinary fields and not harvested early but the crop was tuber indexed to eliminate leafroll and mosaic. In each of the following years, seed was obtained from the same Minnesota grower and compared with the locally propagated seed. Shortly after the recognition of the Minnesota lot as a resistant type, it was discovered that several resistant strains were prevalent in Maine. These strains have been grown in this State for many years. Hence, such strains were compared with the Minnesota lot, and the data are included in tables 2 and 3. While most of the seed used was harvested early, the test plots themselves were not.

TABLE 2.—*Stem-end browning in 1943 in Green Mountain potato strains propagated in Maine for various periods*

Field	Strain or lot	Years grown in Maine	Stem-end browning ^a
			Per cent
1b	Minnesota	First year	3.5
	Minnesota	First year	2.4
	Minnesota	Second year	6.7
	Keswick	Many	20.5
2b	Minnesota	First year	1.9
	Minnesota	Second year	5.8
	Keswick	Many	18.5
3c	Minnesota	First year	20.7
	Minnesota	Second year	22.4
	Highmoor	Many	19.5
	Keswick	Many	54.3
4c	Minnesota	First year	7.5
	Minnesota	Second year	9.1
	Highmoor	Many	11.3
	Keswick	Many	27.8
Average	Minnesota	First year	7.2
	Minnesota	Second year	11.0
	Keswick	Many	30.3

^a After storage at 50° F. for 100 days or more.

^b Each figure for this field is the average of 6 single row plots, each 29 feet long, Latin Square design.

^c Each figure for this field is the average of 8 single row plots, each 29 feet long, Latin Square design.

No great change in susceptibility occurred in four years' time. Furthermore, the figures for the Vermont, Highmoor, and Phillips strains are about the same as those for the Minnesota lot with the possible exception of those for the year it was introduced. Since these strains have been grown in the State as long as or longer than the Keswick strain, it appears very probable that resistance to stem-end browning is a stable characteristic of those strains. The behavior of the Minnesota lot may be a little different. In most cases tubers of this lot had less stem-end browning the year of introduction than did subsequent crops. The differences obtained were usually small but they were consistent. This suggests that the tendency of this lot to produce

stem-end browning is increased slightly by propagation in Maine one or two years but that it does not increase after that. The level of susceptibility reached in that time appears to be identical with that of the Highmoor, Vermont, and Phillips strains. The data are insufficient to determine the cause of this change, although the data on the cage experiment (Table 1) suggest that an insect transmitted agent might be involved.

1943 Tuber Grafts. Eyeless plugs taken from tubers of the Keswick strain (No. 8 cork borer) were grafted into tubers of the Minnesota lot (holes cut with No. 7 cork borer). The holes were cut lengthwise of the tubers so that a longitudinal half could be used as a control. Twenty-seven of the grafted seed pieces together with the corresponding controls were planted

TABLE 3.—*Stem-end browning in Green Mountain potato strains propagated in Maine for various periods*

Year	Field	Strain	Years grown in Maine	Stem-end browning ^a
				<i>Per cent</i>
1944	5	Minnesota	First year	8.1
		Minnesota	Second year ^b	12.2
		Minnesota	Third year	15.2
	6	Minnesota	First year	8.7
		Highmoor	Many	6.9
		Vermont	Many	9.7
	7	Minnesota	First year	6.1
		Vermont	Many	7.5
		Phillips	Many	7.0
1945	8	Minnesota	First year	7.2
		Minnesota	Second year	10.7
		Minnesota	Fourth year	10.6
		Highmoor	Many	9.1
		Keswick	Many	31.6
	9	Minnesota	Fourth year	9.4
		Highmoor	Many	9.9
		Keswick	Many	36.6

^a After storage at 50° F. for 100 days or more. Each figure is the average of 5 single row plots, each 29 feet long, Latin Square design.

^b The seed used for this sample was not harvested early but it was tuber indexed. All other seed was harvested early.

under a field cage. Seven were lost because of seed piece rot or virus disease. In digging, the units were kept separate. The tubers produced by nine of the grafted units developed more stem-end browning than did those of the corresponding Minnesota control (Table 4). The amount of disease in the tubers from all grafted units was intermediate between that in the two controls, corresponding with what would be expected with a 40 per cent "take."³ The nine units where a "take" was indicated gave fully as much stem-end browning as did the susceptible strain used for scions. These nine, as well as the corresponding controls were replanted in 1944. The tubers produced

³ In view of other data presented, the assumption was made that in those cases where a "take" occurred, the stock was free of the virus causing or favoring stem-end browning. Where no "take" occurred, the presence of a mild strain of the virus was assumed.

by eight of these were dug and examined for stem-end browning. All of the replanted grafted units gave higher stem-end browning readings than did their corresponding Minnesota controls, but in three cases the increase was small. The figures for the other five grafted units were almost as high as were those for the Keswick strain.

Others of the tuber grafts made in 1943 were planted in an open field together with their corresponding controls. The units were not kept separate, hence the figures in table 5 are plot averages. The samples from the plots planted with the grafted seed pieces developed an amount of stem-end browning intermediate between that in samples from the two controls. One-third of the crop from each type of seed was replanted in 1944, and again the stem-end browning content of the progeny of the grafted seed pieces was

TABLE 4.—*Transmission by tuber grafts of a factor causing increased development of stem-end browning*

Data recorded	Keswick control	Grafted	Minnesota control
A. No. of units dug, 1943	20	20	20
B. Percentage stem-end browning, 1943	21.8	12.6	3.9
C. No. of units showing increase over Minnesota control, 1943		9	
D. Percentage stem-end browning in C and corresponding controls	24.1	26.7	4.7
E. No. replanted C units and controls dug, 1944	8	8	8
F. Percentage stem-end browning in all units, 1944	40.3	29.8	8.3
G. No. replanted units showing some increase over Minnesota control, 1944		8	
H. No. replanted units showing very definite increase over Minnesota control		5	
I. Percentage stem-end browning in H and corresponding controls	43.3	36.9	11.5

intermediate. In both years, the crop from the grafted units had approximately the amount of stem-end browning that would be expected from a 25 per cent "take."⁴

1944 Tuber Grafts. About 100 new tuber grafts were made in 1944. The average figure on stem-end browning for the entire group was no greater than that for the Minnesota controls. Only ten of the grafted units produced tubers that developed as much stem-end browning as did those from the Keswick controls. The figures for 19 others were considerably higher than those for the Minnesota controls but less than those for the Keswick controls. Of these 29 units, 22 were free of net necrosis and were replanted in 1945, together with the corresponding controls. The progeny of only five of the grafted seed pieces contained an amount of stem-end browning similar to that in the progeny of the Keswick controls. These same five units were among those that had a high percentage of diseased tubers the previous year.

⁴ Calculations from the 1943 data were made after making the assumptions outlined in footnote 3. All of the 1944 tubers were assumed to be infected with either a mild or a severe strain of the virus in question.

Hence, transmission of a factor favoring stem-end browning obtained in only about 5 per cent of the attempted grafts.

1941 Inarch Grafts. In 1944, transmission was attempted by means of inarch grafting. Tubers from the Minnesota lot were planted by tuber units (4 seed pieces per tuber). Two of the hills were left as controls and the other two used for grafting. Tubers of the Keswick strain were planted in 6-inch pots and the plants left in the greenhouse until used. When the field plants were 10 to 12 inches high, the Keswick plants were grafted to a stalk of the field-grown (Minnesota) plants by inarching. The field-grown hills were not trimmed to one stalk. Approximately 2 weeks later the stalk of the potted plant (Keswick) was severed below the graft. Several days later the plants were examined and only those where the Keswick scion remained green and turgid were marked for digging. In the year the grafts were

TABLE 5.—*Transmission by tuber grafts of a factor causing increased development of stem-end browning*

Type of seed used ^a	Stem-end browning	
	1943 ^b	1944 ^c
	<i>Per cent</i>	<i>Per cent</i>
A. Keswick strain	38.6	39.3
B. Minnesota lot	5.9	13.8
C. Eyeless plug of A grafted into a seed piece of B	15.4	21.1

^a A and B seed consisted of unused portions of the tubers that were used for grafting.

^b Average of 6 single row plots, each 29 feet long. Two Latin Squares.

^c Average of 6 single row plots, each 29 feet long. Latin Square design. A representative portion of the 1943 crop was used for seed.

made, the grafted units produced tubers with three times as much stem-end browning as was found in tubers from the corresponding ungrafted units (Table 6). The figures on distribution of diseased tubers in the units indicate that there was a large increase in approximately one-third of the grafted units. In fact, about two-thirds of the grafted units showed some increase over their corresponding control, but in about half of these the increase was small.

Those units free of net necrosis were replanted in 1945 to see if the apparent increase was permanent. It appears that at least part of the increase was temporary, for the progeny of the grafted units produced only 1.4 times as much stem-end browning as the corresponding controls. This, in itself, may not mean too much for it has been shown that the relative amounts of stem-end browning in susceptible and resistant strains vary widely with environmental or seasonal factors (9). Of considerably more significance is the fact that many of the grafted units that had a high percentage of stem-end browning in 1944 also had a high amount in 1945, as shown by items H, I, and J in table 6. In item E, relative to replants from low stem-end browning units, the figure for the grafted units is but little larger than that for the controls, and the majority of the individual units

were low in stem-end browning (items F and G). The figure for replants of grafted units that had high stem-end browning in 1944 was considerably higher than that for the controls in 1945 (item H), and over 60 per cent of the units were high in stem-end browning (items I and J). There was little tendency for the control units which had high stem-end browning the first year to be high in stem-end browning in 1945. The 1944 and 1945 data for

TABLE 6.—*Transmission by inarch grafting of a factor causing increased development of stem-end browning*

Year	Item	Grafted ^a		Not grafted ^b	
		No.	Stem-end browning	No.	Stem end browning
			Per cent		Per cent
1944 ^c	A. All units	115	30.7	115	10.0
	B. Units with 0 to 25 per cent stem end browning	60		100	
	C. Units with over 25 per cent stem-end browning	55		15	
1945 ^d	D. All replants	60	18.8	42	13.1
	E. Replants of B units	24	15.9 ^e	37	12.9
	F. B replants with 0 to 15 per cent stem-end browning	13		25	
	G. B replants with over 15 per cent stem-end browning	11		12	
	H. Replants of C units	36	21.1 ^f	5	16.0
	I. C replants with 0 to 15 per cent stem-end browning	13		4	
	J. C replants with over 15 per cent stem-end browning	23		1	
	K. Total replants with 0 to 15 per cent stem-end browning	26		29	
	L. Total replants with over 15 per cent stem-end browning	34		13	

^a Resistant strain used as stock and susceptible strain as scion.

^b The figures in this column opposite items A and D are for the progeny of seed pieces from the same mother tubers as the grafted units. Those opposite items E and H are for tuber lines classified according to their behavior in 1944. In 1944 the 4 units from one tuber were planted in adjoining hills, and alternate hills were grafted. In 1945 the progeny of grafted and ungrafted units from the same mother tuber were planted in adjacent rows.

^c Average of 6.3 tubers per unit.

^d Average of 76.5 tubers per unit.

^e The controls originating from the same mother tubers as the grafted units had 12.4 per cent stem-end browning.

^f The controls originating from the same mother tubers as the grafted units had 15.9 per cent stem-end browning.

the grafted units gave a coefficient of correlation barely short of significance at the 5 per cent level. This fact does not necessarily indicate lack of correlation, for the sample size in 1944 was small (6.3 tubers per unit) and it has been found that with a given type of strain the individual hills may vary in stem-end browning content from zero to 100 per cent.

Since the grafted hills were not trimmed to one stalk, one would not necessarily expect the progeny of a successful graft to be fully as susceptible as the strain used as scion. This fact plus the fact that all units were not

replanted (because of leafroll infection) makes it difficult to estimate the percentage of successful transmission, but in general it appears that it would be of an order of magnitude of approximately 20 per cent. At least it is clear that in well over 50 per cent of the cases, no evidence of transmission could be detected.

1945 Inarch Grafts. In 1945 the same technique adopted the previous year was used except that both grafted and ungrafted units were trimmed to one stalk. The Highmoor strain, in addition to the Minnesota, was used as stocks. The results are presented in table 7. In the case of the Minnesota lot, grafting resulted in an increase in the average amount of stem-end browning and this was accompanied by a shift toward a larger number of hills with high stem-end browning content. With the Highmoor strain the increase in stem-end browning was small and the shift toward a higher number of high stem-end browning hills was likewise small. Until replantings are made the significance of these results is in doubt but it seems likely that,

TABLE 7.—*Transmission by inarch grafting of a factor causing increased development of stem-end browning. Experiments made in 1945*

Stock	Scion	Average number tubers per hill	Percent-age of stem-end browning	Number of units having following percentages of stem end browning		
				0 to 24	25 to 49	Over 50
Minnesota	Keswick	4.1	25.7	33	15	16
Minnesota	Not grafted	3.6	16.1	42	15	7
Highmoor	Keswick	3.8	25.5	35	26	15
Highmoor	Not grafted	3.4	20.0	43	20	13

at least in the case of the Minnesota seed, some transmission of a factor favoring stem-end browning was accomplished.

Tuber Inoculation. Several attempts were made to transmit the susceptibility factor by inoculating seed pieces of the Minnesota lot. Several methods were tried, including transmission by the cutting knife, by rubbing the two types of seed pieces together, and by dipping the seed piece into the ground pulp of stem-end browning tubers of the Keswick strain followed by bruising of the cut surface by a dull knife. In no case did the progeny of the inoculated seed pieces develop more stem-end browning than did those of the noninoculated pieces. The tubers were replanted the following year but no evidence of transmission was obtained.

Leaf Inoculation. In 1943, cage-grown plants of the Minnesota lot were inoculated with juice of Keswick plants by the leaf mutilation method using carborundum. Only 8.4 per cent of the tubers produced by the inoculated plants developed stem-end browning while 4.3 per cent of those from non-inoculated controls had the disease. This difference was of doubtful significance as the number of tubers was only about 150; consequently the entire crop was replanted in 1944 in well-replicated plots. Ten per cent of the progeny of the tubers from inoculated plants developed stem-end browning

as did 9.9 per cent of that from the controls, indicating no transmission of the factor increasing the amount of stem-end browning. Leaf inoculation to many other species failed to establish the presence of any virus other than latent virus in the Keswick strain.

Stem-end Browning in Tubers Free of Latent Mosaic Virus (Virus X). A Green Mountain seedling was obtained from Dr. E. S. Schultz and examined for stem-end browning. Three tubers were found that had what appeared to be typical stem-end browning. Unfortunately there is no specific test for this disease, but the symptoms in these tubers were indistinguishable from stem-end browning in Green Mountain tubers. These tubers were tested for latent virus both by inoculation to peppers and to *Datura* and by serological methods. Portions of the tubers were planted in the greenhouse, and the plants so produced were tested for the presence of latent mosaic virus (virus X) both by inoculation and by serological methods. In all cases the results were negative. Parallel tests with Green Mountains that contained latent mosaic virus were uniformly positive. Consequently it was concluded that stem-end browning can occur in the absence of latent virus and, hence, that this virus is not the cause of the disease. Further evidence in support of this conclusion is the fact that the symptomatology on Turkish tobacco and *Datura* plants of the latent mosaic virus obtained from the Minnesota lot was indistinguishable from that of the latent mosaic virus from the Keswick strain.

Other Characteristics of Resistant and Susceptible Strains. Under some growing conditions or at least on some fields in some seasons, the resistant strains, especially that from Minnesota, have certain growth characteristics that distinguish them from the susceptible or Keswick strain. The former are sometimes taller, more erect, slightly lighter green; and their upper leaves are slightly more cupped. Their appearance resembles, in general, that of giant hill Green Mountains. These characteristics are not always apparent and in some seasons it is not possible to distinguish between the two types. That the resistant strains do not contain giant hill is indicated by the fact that on the average they produce more tubers per unit yield than the Keswick strain. No other difference has been noted. The two types of strains mature at about the same time and on the average yield equally well. Both are equally susceptible to leafroll infection and the development of net necrosis.

DISCUSSION

From the data presented in this report it is suggested that the Keswick strain of Green Mountain potato contains a virus or virus strain not present in the strains less subject to stem-end browning. The data are not sufficient to determine whether or not a virus is the actual cause of the disease. If such is the case, then it would be necessary to postulate the presence of different strains of the same virus in the two types of stocks. There are several facts that strongly suggest that such is the case. In all of the inarch grafts reported, organic union between stock and scion took place, for after removal

of the root and lower stem of the plant used as scion, the portion above the graft remained green and succulent. Once organic union is established, virus transmission readily takes place in the case of most viruses. If a plant contains one strain of a virus it is usually immune from another strain of the same virus. If the transmission is attempted by grafting, however, protection may be incomplete (1, 8). Hence, the results obtained are consistent with the idea that some of the Minnesota tubers were free of the virus in question, that the remaining ones were infected with a mild strain of the virus, and that the Keswick strain of Green Mountains carried a virulent strain of the virus. The other data support this view. There was a small but consistent tendency for the Minnesota lot to increase in proclivity to stem-end browning development the first year after introduction into Maine. On the average, this increase was of the order of magnitude of 30 to 50 per cent (Tables 2 and 3), suggesting that this percentage of the tubers were virus free when introduced but became infected during the first season in Maine. These figures agree very well with the percentage of grafted units that gave evidence of the transmission of an agent causing increased development of stem-end browning. That the Minnesota lot was not entirely free of the causal agent is indicated by the presence of some stem-end browning in the crop produced under a cage. That it was partially infected is indicated by the fact that the field-grown crop was 40 per cent higher in diseased tubers than was the cage-grown crop. The fact that the 30 to 50 per cent increase in stem-end browning usually noted after one season in Maine was delayed a second season where the crop was produced under a cage is of particular interest. A plausible explanation is that this increase is due to the transmission of a mild strain of the virus to 30 to 50 per cent of the units and that such transmission is prevented by exclusion of insects. The failure of the more virulent virus strain to spread to newly-introduced, virus-free potatoes is not necessarily in disagreement, for there are known cases where different strains of the same virus have different specific insect vectors (2, 10).

SUMMARY

A Green Mountain potato strain that normally produces a crop in which only a few tubers are affected with stem-end browning was made much more subject to development of the disease by tuber grafting and by inarch grafting. Tubers or plants of another strain, the tubers of which are highly predisposed to the disease, were used as scion material. The increased tendency toward stem-end-browning development was apparent not only the year the grafts were made but also in the progeny of the tubers produced by the grafted plants or seed pieces. Transmission was not obtained in all cases where organic union of the grafted plants occurred, nor was it obtained by mechanical inoculation methods. The tubers of a lot from another state became slightly more prone to the development of stem-end browning during the first year or two in Maine under field conditions but not when cage-grown. Otherwise the two types of Green Mountain strains are stable in

that there is no change from year to year in the relative amounts of stem-end browning developed in the crops of each.

The data are interpreted as indicative of the presence of a virus or virus strain in those lots highly subject to the disease and the absence of such a virus or virus strain in the other type. The hypothesis is advanced that a virus is the cause of stem-end browning and that different strains of the virus account for the difference in potentiality for stem-end-browning development evident in different commercial strains of the Green Mountain potato variety.

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HEMP SEED TREATMENTS IN RELATION TO DIFFERENT DOSAGES AND CONDITIONS OF STORAGE

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Striking benefits in stand from the treatment of hemp seed were reported by Robinson.² This was a cooperative enterprise participated in by four state experiment stations and the U. S. Department of Agriculture, and was stimulated by the war needs for hemp. It seemed desirable that more be known about the efficacy of the more promising disinfectants, most effective rate of application, and the result when treated seed is stored for some time before planting. Although war needs are past, hemp has been and no doubt will continue to be grown in peace time. Furthermore, the results are of interest with regard to the treatment of seed in general.

MATERIALS AND METHODS

Hemp seed, *Cannabis sativa*, was obtained from Kentucky through the Hemp Division of the Commodity Credit Corporation, U. S. Department of Agriculture. A shipment of seed of the 1942 crop had a moisture content of 9.4 per cent which is unusually high for hemp. The germination on the seed information tag was given as 90 per cent. The seed was thoroughly mixed, and as hemp seed becomes very dusty from handling, the seed was recleaned with a fanning mill. Part of the seed was adjusted to a higher moisture, and the treatments were made a week later. Germination tests of the same seed lots were made 1 day, 3 months, and 7 months after treating with the disinfectants and dosages at the temperatures and moisture contents given in table 1. Another set containing 14.3 per cent moisture was stored at 70° F., but this became badly molded and was dead after 3 months, and therefore, those data are omitted. Seed of the 1943 crop from the same source had a moisture content of 7.6 per cent and was rated as 90 per cent germination on the seed information tag. Again part of the seed was modulated to a higher moisture content, and germination of the treated seed was tested after 1 day, 3 months, and 7 months storage at the temperatures and moisture contents given in table 2.

The moisture content of part of the seed was raised by adding water to the seed in a container that could be sealed, and then mixing well by shaking it once every day for a week. Treatments were made by tumbling a pint of seed with the disinfectant in a quart jar by means of a machine. Three commercial seed disinfectants were used: Arasan (50 per cent tetramethyl thiuramdisulphide), New Improved Ceresan (5 per cent ethyl mercury phosphate), and Spergon (98 per cent tetrachloro parabenzoquinone). Each one was used at 2 or 3 dosages, as shown in tables 1 and 2. After treatment, the

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² Robinson, Brittain B. Greenhouse seed treatment studies on hemp. Jour. Amer. Soc. Agron. 35: 911-914. 1943.

seed was stored in sealed pint jars filled nearly full. In this manner it was possible to keep the moisture constant. A recheck of the moisture content after 7 months' storage showed that the moistures were about the same as at the beginning except in the case of the moldy seed, in which moisture had risen from 14.3 to 14.6 per cent.

TABLE 1.—*Germination of hemp seed of two moisture contents after treating with three seed disinfectants and storing at 32° and 70° F. Experiment 1, Urbana, Illinois, 1943*

Storage temperature	Grain moisture	Seed disinfectant used for treating	Rate of application	Time of planting after treatment		
				1 day	3 mo.	7 mo.
32° F.	Pct. 9.4			Pct.	Pct.	Pct.
		None		57.0	49.3	29.5
		Arasan	0.5	69.6	64.0	47.3
		Do	1	68.0	64.3	50.8
		Do	2	70.0	60.0	55.0
		N.I. Ceresan	0.5	73.3	57.5	52.3
		Do	1	76.5	65.8	63.0
		Do	2	78.6	70.5	63.0
		Spergon	1.5	73.6	60.0	50.3
		Do	3	72.7	65.8	60.5
		Least Significant Difference (5 per cent level)		10.5	9.0	12.1
32° F.	14.3	None		55.8	45.3	33.3
		Arasan	0.5	65.7	60.8	32.0
		Do	1	72.0	60.5	44.0
		Do	2	73.5	61.5	47.8
		N.I. Ceresan	0.5	72.7	74.5	70.8
		Do	1	77.3	53.8	43.3
		Do	2	77.2	49.5	36.0
		Spergon	1.5	68.3	49.8	52.3
		Do	3	69.4	52.0	49.8
		Least Significant Difference		9.7	10.6	9.9
70° F.	9.4	None		56.3	43.8	9.9
		Arasan	0.5	67.9	58.8	24.3
		Do	1	69.5	53.0	24.0
		Do	2	71.1	57.3	27.8
		N.I. Ceresan	0.5	69.0	52.8	37.8
		Do	1	78.4	57.8	48.8
		Do	2	75.2	57.0	51.8
		Spergon	1.5	70.9	45.5	33.0
		Do	3	73.1	51.5	34.3
		Least Significant Difference		12.7	10.3	9.4

The tests were made in greenhouse benches filled with dark colored soil brought from the University Farm. The greenhouse was kept at 65° to 70° F., as far as possible, but occasionally the temperatures went higher. The experiments were timed so that no tests were made during the hot summer months. Each test consisted of 4 to 6 randomized replications of 100 kernels for each item.

TABLE 2.—*Germination of hemp seed of two moisture contents after treating with three seed disinfectants and storing at 70° and 90° F. Experiment 2, Urbana, Illinois, 1944*

Storage temperature	Grain moisture	Seed disinfectant used for treating	Rate of application	Time of planting after treatment		
				1 day	3 mo.	7 mo.
	<i>Pct.</i>		<i>Oz./Bu.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>
70° F.	7.6	None		72.7	69.8	65.7
		Arasan	0.5	91.0	89.0	85.3
		Do	1	93.3	90.0	88.2
		Do	2	92.0	90.5	89.7
		N.I. Ceresan	0.5	93.3	88.5	86.3
		Do	1	92.2	91.0	88.5
		Do	2	94.5	90.3	86.8
		Spergon	1.5	84.5	84.3	80.7
		Do	3	85.7	83.0	84.0
		Least Significant Difference (5 per cent level)				7.5
70° F.	10.1	None		67.0	61.0	47.2
		Arasan	0.5	81.8	77.5	59.8
		Do	1	86.0	79.0	64.0
		Do	2	90.2	79.3	61.0
		N.I. Ceresan	0.5	89.8	78.5	72.8
		Do	1	92.8	71.5	69.7
		Do	2	93.5	63.8	34.0
		Spergon	1.5	74.5	74.8	64.5
		Do	3	73.3	70.8	62.5
		Least Significant Difference				10.3
90° F.	7.6	None		72.7	65.3	50.5
		Arasan	0.5	91.0	86.5	63.8
		Do	1	93.3	85.8	67.0
		Do	2	92.0	87.5	73.8
		N.I. Ceresan	0.5	93.3	87.5	81.5
		Do	1	92.2	87.3	79.7
		Do	2	94.5	85.5	77.7
		Spergon	1.5	84.5	83.5	65.2
		Do	3	85.7	85.3	66.0
		Least Significant Difference				7.5
90° F.	10.1	None		67.0	43.5	0
		Arasan	0.5	81.8	55.3	0
		Do	1	86.0	52.0	0
		Do	2	90.2	59.5	0
		N.I. Ceresan	0.5	89.8	66.8	0
		Do	1	92.8	67.0	0
		Do	2	93.5	33.8	0
		Spergon	1.5	74.5	50.0	0
		Do	3	73.3	53.0	0
		Least Significant Difference				10.3

RESULTS

Striking increases in stand from treatment were obtained in both years with the three disinfectants used. This was due primarily to the preven-

tion of pre-emergence seedling blight. Some damping-off occurred also, but no significant differences were caused by seed treatment.

Seed Deterioration

The seed deteriorated very much in viability during 7 months' storage, especially that used in Experiment 1, which was low in vitality at the start. Seed deteriorated even when stored at 32° F. At this low temperature, the seed moisture, whether 9.4 or 14.3 per cent, made no apparent difference. In both cases the stand from the nontreated seed was reduced approximately half after 7 months' storage. When considering the average results from the use of the three seed disinfectants, at the most favorable dosage of each, the stand from treated seed was considerably higher than for the nontreated seed at the start; and after 7 months' storage the reduction in stand with treated seed was approximately 20 per cent.

When the seed was stored at 70° F. (Experiment 1), and the grain moisture was 9.4 per cent, deterioration in vitality was much more rapid than at 32° F. The reduction in stand in 7 months' storage time was 82 per cent when nontreated seed was used. In the case of treated seed, there was an average reduction of 48 per cent. When the grain moisture was 14.3 per cent, at 70° F. the grain was dead after 3 months and more or less moldy in spite of the treatments with disinfectants.

In Experiment 2, the grain moistures were lower, and one of the storage temperatures was higher than in Experiment 1. Initial germination was somewhat better, and deterioration in vitality was not so extreme in 7 months' time. In Experiment 2, which involved storage temperatures of 70° and 90° F. and grain moistures of 7.6 and 10.1 per cent, deterioration was least at the lowest temperature and moisture, and became greater with rise of either temperature or moisture. At the highest temperature and moisture, the seed germinated poorly after 3 months and was dead after 7 months' storage. Again, the lower the vitality of the seed, the more the stand was benefited by seed treatment.

Dosage

The only damage from too high a dosage occurred with New Improved Ceresan when used with seed at the higher moistures. This was true in Experiment 1 with grain moisture of 14.3 per cent, even though the storage temperature was only 32° F., and in Experiment 2 with grain moisture at 10.1 per cent and storage at 70° and 90° F. The injury was pronounced after 3 months' storage and worse after 7 months. The use of only $\frac{1}{2}$ oz. per bushel of New Improved Ceresan caused no injury under any of the experimental conditions. However, with seed having only 7.6 and 9.4 per cent moisture, the higher rates, for the most part, gave the best protection.

Arasan used at $\frac{1}{2}$, 1, and 2 oz. per bushel gave, in general, progressively better protection as the dosage was increased, irrespective of the temperature or grain moisture during storage. Spergon also had a tendency to give

better results when used at 3 oz. per bushel than when used at $1\frac{1}{2}$ oz., irrespective of temperature or moisture.

If one keeps in mind the possibility of damage from a high dosage of New Improved Ceresan under some conditions, 1 oz. per bushel appears to be a good recommendation. Arasan was used to best advantage at 2 oz. per bushel. Spergon should probably be recommended at about 2 oz. per bushel. From the data in both experiments, New Improved Ceresan appeared to be most effective, Arasan second, and Spergon third. The differences, however, were not great and all of the treatments were beneficial.

DISCUSSION

The cost of hemp seed is much higher than that of the small grains, and the seeding rate in bushels per acre is about the same as for wheat. Therefore, the saving of seed, through obtaining better stands, is a very important

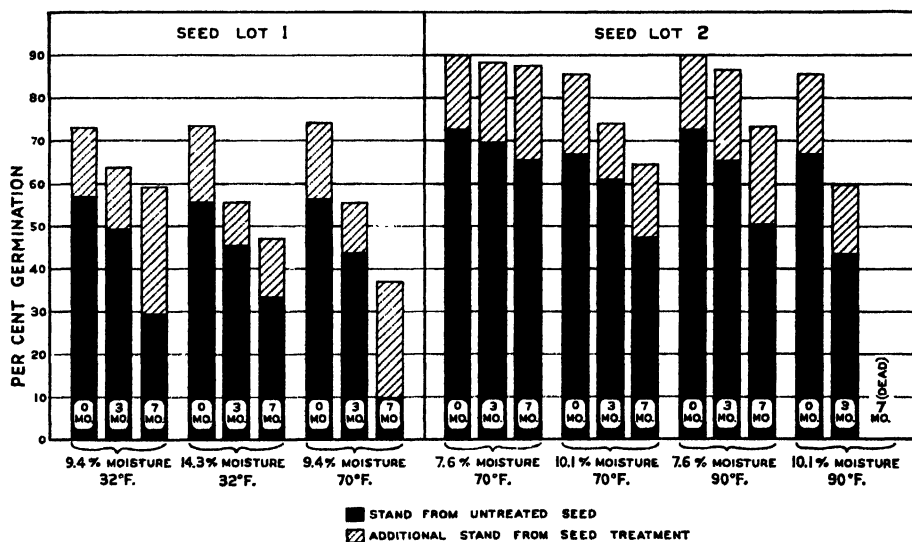


FIG. 1. Germination percentages of two lots of hemp seed when treated seed was held at several different moistures and temperatures for 0, 3, and 7 months before planting. Data are the averages for Arasan, New Improved Ceresan, and Spergon, used at the most favorable dosage.

consideration. If the $1\frac{1}{2}$ bushel-per-acre rate ordinarily used is satisfactory, then one bushel of treated seed should give at least as good stands according to these experiments. On the other hand, seed may be poor in vitality, unknown to the farmer, and if seed treatment is not used the poor stands obtained will result in coarse stalks which make a poor grade of fiber.

The black bars in figure 1 show graphically the drop in vitality as the seed ages. The planting after 3 months' storage was made in spring at approximately normal planting time, while the 7 months' storage lots were planted in September. None of the seed was stored long enough to be equivalent to seed carried over to the following year. The cross-hatched

part of the bars shows also that seed with poorest vitality benefited most from seed treatment. Therefore, seed carried over for an additional year would seem to be in special need of protection by a fungicide.

Of general interest for seed treatment knowledge of farm crops is the corroboration of existing fragmentary information that doses of Arasan, higher than ordinarily recommended, probably up to the amount that will stick to the seed appear to be harmless to seed vitality. With Ceresan, on the other hand, there is danger from an overdosage, and this is influenced to a considerable extent by the moisture content of the seed treated.

SUMMARY

Two seed lots of hemp were treated with Arasan and New Improved Ceresan at the rates of $\frac{1}{2}$, 1, and 2 oz. per bushel, and with Spergon at $1\frac{1}{2}$ and 3 oz. per bushel.

Grain moistures of 9.4 and 14.3 per cent and storage temperatures of 32° and 70° F. were used in 1943, and moistures of 7.6 and 10.1 per cent and storage temperatures of 70° and 90° F. were used in 1944. Storage periods from the time of treating until planting were 1 day, 3 months, and 7 months.

Seed vitality deteriorated rapidly in storage, even at 32° F. At 32° F. the differences in seed moisture had no apparent effect, but at higher temperatures, seed with the most moisture deteriorated most rapidly.

All three seed disinfectants produced striking increases in stand; the poorer the vitality of the seed, the greater the benefit, up to 400 per cent increase over the nontreated seed.

The maximum rate of application of Arasan and Spergon gave better average results than lower rates, and there was no indication of injury to seed vitality at any of the seed moistures or storage temperatures used. New Improved Ceresan, on the other hand, caused considerable injury when used at 2 oz. per bushel on seed that contained 10.1 or 14.3 per cent moisture and when such treated seed was stored for 3 or 7 months at 32° to 90° F. With grain moistures of 7.6 and 9.4 per cent, there was no apparent damage, and 2 oz. per bushel gave the best average stand.

From these experiments it would appear that for treating hemp seed, New Improved Ceresan should be recommended at the rate of about 1 oz. per bushel, while Arasan and Spergon should be used at about 2 oz. per bushel.

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BACTERIAL SPOT OF HONEYDEW MELON

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Honeydew melons (*Cucumis melo* L. var. *inodorus* Naud.) bearing numerous water-soaked spots were first observed by G. B. Ramsey in a shipment from Colorado in September, 1940. Although cultures from these spots yielded bacteria in most instances, no attempts were made to identify the organisms.

On September 21, 1944, the Food Products Inspection Service at Chicago, Illinois, reported that in one carlot of honeydew melons from Colorado, 40 per cent bore sunken, water-soaked spots affecting from 10 to 75 per cent of the surface of the melons. Examination in the laboratory showed the diseased areas to be slightly sunken, circular to oblong, water-soaked, greenish-tan, and 2 to 6 mm. in diameter. On some melons the lesions had so coalesced that individual spots were indistinguishable (Fig. 1, A). Such areas were brown to black. Paraffin sections made from sunken, water-soaked lesions showed that the bacteria had entered the fruit through stomata and were between the parenchyma cells beneath (Fig. 1, B and C).²

Pure cultures were readily obtained by the dilution-plate and tissue-plant methods. Preliminary studies showed the organism to be motile by one to three polar flagella, rod-shaped with rounded ends, and Gram-negative. When it was grown in beef-extract agar and in asparagin agar, an abundant green-fluorescent pigment was produced. The same disease was again observed in August, 1945, on honeydew melons from Colorado and Arizona. Pure cultures obtained from these melons were identical in their biochemical and physiological reactions with those obtained in the 1944 isolations.

There is no mention in the literature of a similar disease of honeydew melon or even of the common muskmelon (*Cucumis melo* L.).

Carsner (4) in studies on the angular leaf spot of cucumbers was unable to obtain infection on 11 varieties of muskmelon from field or greenhouse inoculations with *Pseudomonas lachrymans* (E. F. Sm. and Bryan) Ferr. Anderson and Thornberry (2) report having observed a severe outbreak of angular leaf spot of cucurbits in Michigan in 1938 on seed cucumbers and a bacterial spot of pumpkin, squash, cantaloupe, and watermelon that closely resembled the angular leaf spot of cucumbers.

The physiological reactions of one of the white isolates (species undetermined) which Anderson and Thornberry obtained from cucumbers³ that

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² Acknowledgment is made of the assistance of Barbara C. Heiberg who prepared the slides from which the photomicrographs were made.

³ Data kindly supplied the writer by H. H. Thornberry.

were infected with a disease resembling angular leaf spot were compared with a number of isolates that the writer consistently obtained from diseased

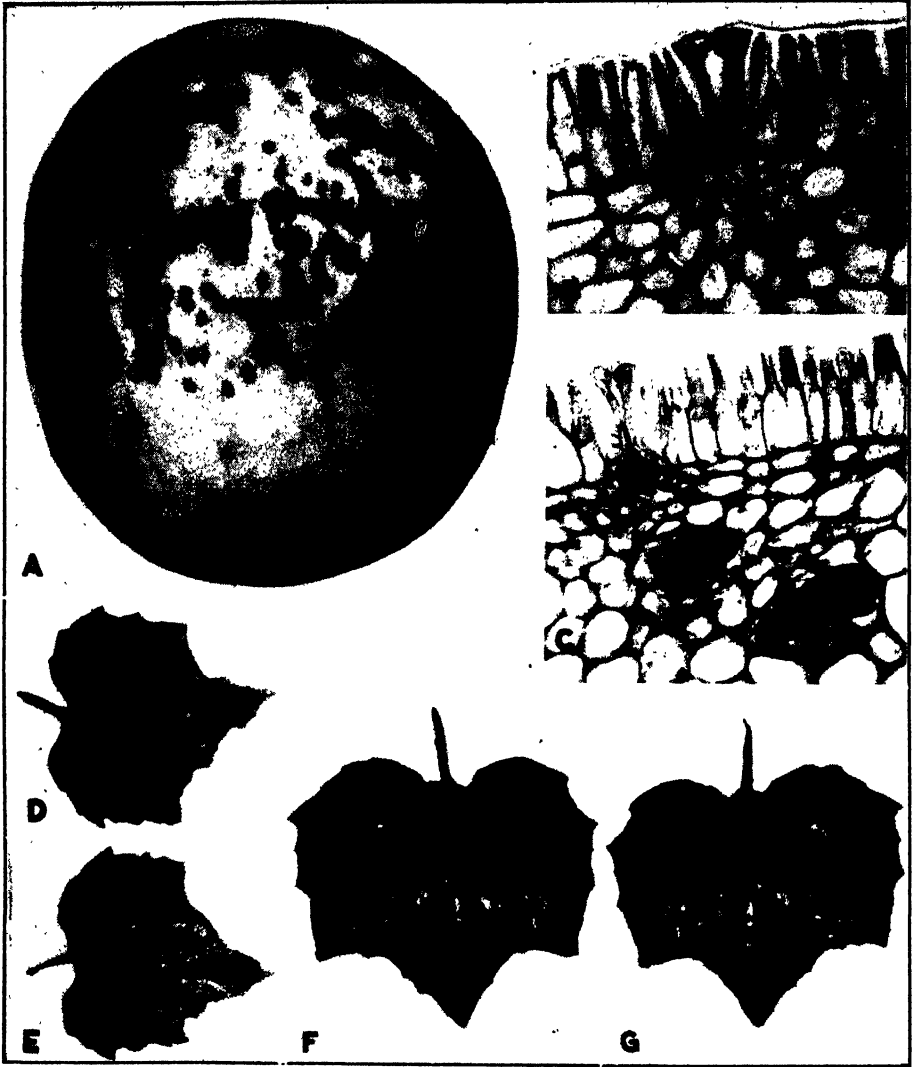


FIG. 1. A. Bacterial infection of honeydew melon. B. Cross section through epidermis of honeydew melon showing a normal stoma. 225 \times . C. Cross section through epidermis of diseased honeydew melon. Bacteria have entered through a stoma and are in tissues beneath. 210 \times . D. Infection of honeydew leaf by spray inoculation with honeydew isolate. E. Infection of cucumber leaf by spray inoculation with honeydew isolate. F. Infection of honeydew leaf 5 days after wound inoculation with *Pseudomonas lachrymans*. G. Infection of cucumber leaf 12 days after spray inoculation with *Pseudomonas lachrymans*.

honeydew melons. The results of the tests indicated that their isolate from cucumbers was distinct from the writer's melon isolates.

In the studies herein described, morphological and cultural character-

istics, biochemical reactions, and pathogenicity of a number of honeydew isolates were determined. The organisms were isolated in pure culture by the dilution plate method. Cultures were maintained in beef-extract agar (pH 6.8). Single colony isolations from cultures purified by the dilution-plate method were used in the studies. Six isolates from diseased honeydew melons were used in the morphological and biochemical studies. In all cases the tests were run in duplicate. Two authentic cultures of *Pseudomonas lachrymans* furnished by the American Type Culture Collection and four cultures of *Pseudomonas lachrymans* from cucumbers from a carlot shipment on the local market were included in the study for comparison.

MORPHOLOGY AND STAINING REACTIONS

Methods. For determination of form and size "negative" preparations from smears of the various honeydew and *Pseudomonas lachrymans* isolates from beef-extract agar were made with one per cent nigrosine. Fisher and Conn's (5) flagella stain was used to determine the number and position of flagella. Gram reaction was determined according to Hucker and Conn's (6) modification of Gram's stain. Ziehl-Nielsen's method was employed for acid fast properties. The Anthony (1) method of capsule staining was employed.

Morphological characters. The honeydew melon isolates are rods with rounded ends, occurring singly. Neither capsules nor spores were demonstrated. The cells measure 1.4 to 3.0 by 0.65 to 1.5 microns. They are readily stained by carbol fuchsin and crystal violet. The organisms are actively motile by one to three polar flagella, generally at one end. They are Gram-negative and not acid fast. In their morphological characteristics *Pseudomonas lachrymans* and the honeydew isolates are indistinguishable.

CULTURAL CHARACTERISTICS

Growth of the honeydew melon organisms and *Pseudomonas lachrymans* on beef-extract agar slants (pH 6.9) is white, flat, and butyrous, the medium becoming greened after three days. On beef-extract dilution plates after six days the colonies are white, slightly raised, circular, transparent, surface smooth, edges irregular. On potato-dextrose agar (pH 6.9) growth is slightly raised, filiform, white, and butyrous. Growth in nutrient broth (pH 6.9) is turbid after 24 hours, becoming greened after 48 hours. On asparagin medium (pH 6.8) growth after four days is white, flat, and butyrous. The medium is greened. Brom-cresol-purple milk becomes bluish purple and alkaline after 10 days, but no curd is formed. Growth of the honeydew organisms and of *Pseudomonas lachrymans* in plain gelatin stabs is evident in three days. Growth is first crateriform, later becoming stratiform. Liquefaction is complete in 21 days.

BIOCHEMICAL REACTIONS

Relation to free oxygen. When grown in Smith fermentation tubes growth is evident first in the open arms and later progresses to the domes indicating that the organism is a facultative anaerobe. There is no gas.

Nitrate reduction. The alpha-naphthylamine-sulfanilic acid test was employed on 1-, 2-, 5-, 7-, 10-, and 14-day-old cultures of the different isolates. The organism *Escherichia coli* was used as a control. Nitrate reduction did not occur in any of the honeydew or *Pseudomonas lachrymans* cultures.

Indole production. The various isolates growing in Bacto-tryptophane broth (pH 6.8) were tested by the Kovaes method (7) for indole production at the end of 7, 14, and 21 days. No positive reaction was obtained with any of the isolates. When *Escherichia coli* was used as a control organism a positive test for indole was obtained.

Ammonia production. Strips of filter paper saturated with a freshly prepared Nessler's solution were hung over 36- and 48-hour-old beef extract broth cultures of the various isolates. A positive test for ammonia was obtained.

Hydrogen sulphide. Strips of lead acetate paper failed to turn black when hung over beef-extract broth cultures of the various isolates, indicating that no hydrogen sulphide was being formed. *Escherichia coli* used as a control organism gave a positive test for hydrogen sulphide.

Hydrolysis of starch. Streak inoculations were made in beef-extract agar containing 0.2 per cent soluble starch. After five days, the surfaces of the plates were flooded with a saturated solution of iodine in 50 per cent alcohol. None showed any clear zone outside the area of growth indicating that no diastatic action had occurred.

The results of the cultural and biochemical tests with the honeydew organism and *Pseudomonas lachrymans* indicate that they are identical.

CARBON METABOLISM

Nine isolates from diseased honeydew melons and two isolates of *Pseudomonas lachrymans* were included in the studies of carbon metabolism. All tests were run in duplicate. Sugars, alcohols, and glucosides were added to the modified synthetic medium of Ayres, Rupp, and Johnson (3) as recommended in the Manual of Methods of Pure Culture Study of Bacteria issued by the Society of American Bacteriologists (8). The basal medium was adjusted to pH 7.0 and sterilized by autoclaving at 15 pounds pressure for 15 minutes. Brom cresol purple at a concentration of 0.02 per cent was added as an indicator. One per cent of some one of the sugars was added to the liquid basal medium before use for culturing. In order to avoid breaking down of the sugars during sterilization, lactose, levulose, maltose, raffinose, and sucrose were sterilized by filtration through a Berkefeld filter. Twenty-seven carbon sources were used. Inoculations were made in duplicate. Cultures were incubated at 27° C. Color change from purple to yellow was considered the indication of fermentation. Final readings were made at the end of 21 days. Organic acid media were prepared according to the Ayres-Rupp-Johnson method (3) except that 0.11 per cent acid was used. Brom thymol blue was used as an indicator. The criterion for fermentation was the presence of growth as indicated by turbidity and an increase in alkalinity.

The honeydew melon isolates and *Pseudomonas lachrymans* cultures utilized arabinose, xylose, sucrose, dextrose, levulose, galactose, mannose, and sorbitol. Rhamnose, maltose, lactose, trehalose, raffinose, melezitose, melibiose, cellubiose, starch, inulin, dextrin, glycogen, inositol, glycerol, dulcitol, erythritol, salicin, esculin, and arbutin were not fermented.

The honeydew melon isolates averaged more rapid fermentation of the carbohydrates than the *Pseudomonas lachrymans* cultures, but as has been stated, the final reactions of all the isolates were the same. The honeydew melon and *Pseudomonas lachrymans* isolates utilized malic, citric, and succinic acids but not acetic, lactic, formic, or tartaric acid. It appears from the foregoing that there are no significant differences in the carbon metabolism of the honeydew melon organism and *Pseudomonas lachrymans* and that they should be considered identical.

PATHOGENICITY

Three mature honeydew melons and 4 cucumber fruits were inoculated by needle puncture with 24-hour-old cultures from infected honeydew melons. Infection, as evidenced by water-soaked areas around needle punctures, was first observed on each of the honeydew melons 5 days after inoculation and on cucumbers 8 days after inoculation. After 11 days the infected areas on the honeydew melons were slightly sunken but there was no appreciable change in color. On cucumber, 11 days after inoculation, infected areas were slightly water-soaked and yellowish. Control melons and cucumbers inoculated by sterile needle punctures remained free of the disease. The organism was readily recovered in pure culture from the infected areas and these reisolates were used in further inoculation experiments.

In another experiment 2 mature honeydew melons and 4 mature cucumbers obtained on the local market were inoculated by spraying the uninjured surfaces with a water suspension of a 24-hour-old culture of one of the honeydew isolates. The cucumbers and melons were observed for 15 days. No infection was apparent during that time. A 24-hour-old culture of *Pseudomonas lachrymans* was used in spraying the uninjured surfaces of 2 honeydew melons and 4 mature cucumbers. No infection was apparent 15 days later. Uninjured melons and cucumbers inoculated with sterile water remained free of the disease.

Two mature honeydew melons and 3 cucumber fruits were inoculated by needle puncture with a 24-hour-old culture of *Pseudomonas lachrymans*. Infection of cucumbers was first observed 9 days after inoculation. After 15 days the infected area was very slightly sunken and light yellow. Infection of honeydew melons was noted 8 days after inoculation. After 15 days the infected area was sunken and yellowish-brown. Reisolutions from infected honeydew melons and cucumbers were readily made. Control melons and cucumbers inoculated by sterile needle puncture remained free of the disease.

In greenhouse inoculations one series of 6 young honeydew melon and

6 cucumber plants growing in pots were sprayed with a suspension of a 24-hour-old culture of one of the honeydew isolates. A second series of 6 melons and 6 cucumber plants was sprayed with a suspension of a 24-hour-old culture of *Pseudomonas lachrymans*. After inoculation the plants were placed under bell jars for 24 hours after which they were returned to the greenhouse bench and held at 21° C. Infection was evident within 72 hours on foliage of the melon and cucumber plants inoculated with either the honeydew isolate or *Pseudomonas lachrymans*. Six days after inoculation the melon foliage inoculated with the honeydew isolate was so heavily infected that many lesions had coalesced (Fig. 1, D). Infection of cucumber leaves, inoculated with the honeydew organism or with *Pseudomonas lachrymans*, was moderate at the end of three days and showed considerable further spread at the end of six days though it was not so severe as on the melon plants. At this stage the lesions varied from mere dots to areas 1 to 2 mm. in diameter on each host when either the honeydew organism or *Pseudomonas lachrymans* was used (Fig. 1, F and G). Some of the lesions were more or less irregular and water-soaked. When dried, infected leaves were examined, the infected areas appeared chalky. The causal organisms were reisolated by plating from these lesions.

In another experiment 10 honeydew melon and 10 cucumber plants were used for wound inoculations. Four each of the melon and cucumber plants were inoculated by needle punctures into leaves, petioles, and stems with 24-hour-old agar slant cultures of *Pseudomonas lachrymans* and one of the honeydew isolates. Two of the melons and two of the cucumbers injured with a sterile needle, served as controls. Infection was evident within 48 hours on foliage of the melons and cucumbers inoculated by needle punctures with either the melon isolate or *Pseudomonas lachrymans* (Fig. 1, E). Petiole and stem infections were apparent 4 days after inoculation. Wilting was observed with both hosts but was much more rapid in the honeydew melon plants inoculated with the honeydew melon isolate. Melon and cucumber plants injured by sterile needle punctures remained free of the disease.

The foregoing inoculation tests show that the honeydew melon isolates and *Pseudomonas lachrymans* are pathogenic to fruits of honeydew melon and cucumber when needle puncture inoculations are made and to honeydew melon and cucumber plants inoculated by spraying with a bacterial suspension or with wounding. Because of the pathogenic similarity it is concluded that the melon organism should be considered identical with *Pseudomonas lachrymans*.

SUMMARY

A hitherto unreported bacterial disease of honeydew melons is described. A comparison of the morphological characteristics of the isolates from diseased honeydew melons with *Pseudomonas lachrymans* indicates that they are indistinguishable morphologically. The results of cultural and biochemical tests with the honeydew melon organism and *Pseudomonas lachry-*

mans indicate that they are identical. Studies have shown that there are no significant differences in carbon metabolism of the honeydew melon organism and *Pseudomonas lachrymans*. Pathogenicity studies show that both organisms are pathogenic to fruits, leaves, and stems of honeydew melon and cucumber. It is suggested, as a result of these studies, that the honeydew melon organism should be considered identical with *Pseudomonas lachrymans*.

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A NEW RACE OF *CERCOSPORA ORYZAE* ON RICE¹

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(Accepted for publication August 24, 1946)

Five races and several subraces of *Cercospora oryzae* Miyake were reported by Ryker³ in 1943 as occurring on rice in Louisiana. These races were differentiated by their differing range of pathogenicity on the following rice varieties: Blue Rose, Blue Rose 41, Fortuna, Caloro, Colusa, Zenith, Delitus, and Southern Red rice. Before 1944, the variety Rexoro had been resistant to all of these races both in commercial fields and in experimental plots artificially inoculated. In 1944, however, and again in 1945, Rexoro was susceptible both in Texas⁴ and in Louisiana.⁵⁻⁶

Texas Patna and Bluebonnet, two new rice varieties of which Rexoro was one of the parents, also were susceptible. In 1945, the disease was widespread on Rexoro throughout the rice area of Texas and Louisiana. The fungus was isolated from Rexoro and Texas Patna and used to inoculate differential varieties. The results substantiated the indications that the fungus on Rexoro and Texas Patna is a new race of *Cercospora oryzae*. It is the purpose of the present paper to report the disease reactions of the differential varieties used and to summarize the results of artificial inoculations with the new race on commercial varieties and varieties and selections introduced from foreign countries, so that the information may be available for use in breeding for resistance to this new race. This information is particularly important because Rexoro was the most important high-quality, long-grain variety of rice grown in the United States in 1945.

MATERIALS AND METHODS

From a number of cultures isolated from lesions on plants of Rexoro and Texas Patna, one culture from Texas Patna from Texas and one from Rexoro from Louisiana were used in June to July to inoculate young rice plants of 6 differential varieties and 24 commercial varieties growing in rod rows at the Rice Experiment Station, Crowley, La., and at Texas Substation No. 4, Beaumont, Texas. At the latter station, 703 foreign varieties and selections from various parts of the world also were similarly inoculated with the culture of the fungus isolated from Texas Patna.

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³ Ryker, T. C. Physiologic specialization in *Cercospora oryzae*. *Phytopath.* **33**: 70-74. 1943.

⁴ Tullis, E. C., and G. E. Altstatt. Rice diseases in Texas in 1944. U. S. Dept. Agr., *Pl. Dis. Repr.* **28**: 1103-1104. 1944.

⁵ Chilton, S. J. P. A new race of *Cercospora oryzae*. *La. Agr. Expt. Sta. Ann. Rept.* 1943-1944: 112. 1945.

⁶ Chilton, S. J. P., and E. C. Tullis. A new race of *Cercospora oryzae* on rice. (*Abstr.*) *Phytopath.* **36**: 395. 1946.

The method of inoculation was the same as that described by Ryker³ as his method No. 2. That is, spore suspensions from pure cultures were sprayed on the young rice plants in the evening between six o'clock and dark.

The ratings used to record the disease reaction, that is the susceptibility or resistance of the various varieties, were: S = susceptible, MR = moderately resistant, and R = resistant. The susceptibility or resistance of a variety was determined (1) by the length of time elapsed after the inoculation before the lesions began to appear, and (2) by the size of the lesions when fully developed. On susceptible varieties, lesions began to appear 10 to 12 days after inoculation and usually were 7–10 mm. long at the end of three weeks. The moderately resistant varieties reacted similarly except that the incubation period was two or three days longer and the lesions were shorter. On resistant varieties, lesions did not appear until about 18 days or more after inoculation, and they remained small.

REACTIONS OF DIFFERENTIAL VARIETIES

The disease reactions of the six differential varieties to the previously described races 1 through 5 and to the new race, designated as race 6, are given in table 1.

TABLE 1.—*Disease reaction of differential varieties to six races of Cercospora oryzae*

Variety	Reaction to					
	Race 1	Race 2	Race 3	Race 4	Race 5	Race 6
Blue Rose	S	MR	S	R	R	R
Blue Rose 41	R	S	R	R	R	R
Caloro	R	R	S	R	R	R
Fortuna	R	R	R	S	R	R
Red Rice	R	R	R	R	S	
Rexoro	R	R	R	R	R	S

Rexoro is resistant to races 1 through 5, and it is the only one of the differential varieties that is susceptible to the new race 6.

RELATIVE RESISTANCE OF VARIETIES

The commercial varieties of rice found to be resistant to race 6 include (1) the short-grain: Acadia, Asahi, Caloro, Cody, and Colusa; (2) the medium-grain: Arkrose, Blue Rose, Blue Rose 41, Calady 40, Early Prolific, Hill Medium, Kamrose, Magnolia, and Zenith; and (3) the long-grain: Arkansas Fortuna, Delrex, Fortuna, Lady Wright, Nira, Nira 43, and Prelude.

The commercial varieties susceptible to race 6 were all long-grain: Bluebonnet, Rexoro, and Texas Patna. Bluebonnet, Texas Patna, and a selection known as Nirex, which also was susceptible, were developed from crosses in which Rexoro was one of the parents.

Of the 703 foreign varieties and selections, 555 were resistant to race 6, while 109 were moderately resistant and 16 were susceptible. The other 23 appeared to be segregating. Among the 555 foreign varieties and selections resistant to race 6, were the following glutinous varieties, some of which may be found of value in California: Asahi mochi, Bozu mochi, Myogahari mochi, and Mochi Gomi.

DISCUSSION

The rice varieties Bluebonnet, Texas Patna, and Nirex, all susceptible to race 6, were developed from crosses in which Rexoro was one of the parents. Their susceptibility to this race clearly came from Rexoro, the female parent, as both Fortuna, the male parent of Bluebonnet, and Nira, the male parent of Nirex, are resistant to race 6. The reaction of the male parent of Texas Patna, C.I. 5094, to race 6 is not known, but presumably it is resistant. Likewise, the reaction of Delitus, which unfortunately was not included in the tests, is thought to be resistant, because Delrex, which was developed from a cross between Rexoro and Delitus, is resistant. That is, the Delitus parent undoubtedly carried the factor for resistance in Delrex. This is the more likely, because, as previously reported,⁹ an F_2 generation in a cross between a resistant variety and Rexoro segregated in a ratio of 3 resistant to 1 susceptible.

SUMMARY

A new race of *Cercospora oryzae*, designated as race 6, is reported on rice in Louisiana and Texas. Race 6 attacks Rexoro and certain other commercial varieties of Rexoro parentage. The reactions of the differential varieties of rice used to separate the various races of *Cercospora oryzae* as well as the reactions of a number of other commercial varieties and foreign introductions are reported.

LOUISIANA AGRICULTURAL EXPERIMENT STATION,

BATON ROUGE, LOUISIANA

AND

RICE EXPERIMENT STATION,

BEAUMONT, TEXAS

THE PREPARATION OF MANUSCRIPTS FOR PHYTOPATHOLOGY¹

A. J. RIKER

WRITING—A PART OF RESEARCH

The publication of results from research is an important matter because of the permanence of the record and its use by others. The manner in which it is done reflects not only upon the individual worker, but also upon the organization of which he is a part. The preparation of a manuscript which accurately conveys readable ideas is an essential phase of research; it is just as valuable as doing more experiments; and it is fully as worthy of our best efforts (cf. Allen, 1). Here stands our brain child. How shall we dress it for presentation to the world?

Writing is one of our most important arts. It crystallizes for us the experiments and ideas of others, living and dead, near at hand and in a far country. It gives us the combined experience of the human mind to use and to pass on, with such additions as we ourselves can make.

Making these additions through printed papers sometimes causes both headaches and heartaches. Recognizing that not every member can ride Pegasus well, The American Phytopathological Society has requested a new (cf. Reddick, 18) series of recommendations on writing manuscripts. It has also cracked the whip over the Editorial Board in a mandate "to sharpen editorial pencils," to employ at least two qualified and anonymous referees on each manuscript, and to improve the quality of papers published through suggestion and encouragement, as far as possible, and to reject papers when necessary.

By this action the Society is not reaching for perfection but is striving to maintain a reasonably high technical standard, to avoid unnecessary burdens on the unpaid editorial staff, to meet the greatly increased cost of printing, and above all to prevent our science from sinking under the very bulk of present-day publication (cf. Weiss, 23). Although the subject is pathological, the report of it need not be pathological too.

Our purposes are to diagnose some of the diseases of manuscripts and, for those who want them, to suggest both prevention and cure.

A skillful investigator avoids rushing into print with an account of "flea" research. By this we mean the kind in which he jumps on this sub-

¹ This article was prepared at the suggestion of the Council of The American Phytopathological Society and at the invitation of the other members of the Editorial Board of Phytopathology. These and various other friends and colleagues, including particularly C. E. Allen, Noble Clark, R. E. Cleland, J. G. Dickson, H. B. Humphrey, G. W. Keitt, Ross Marvin, M. C. Merrill, and J. C. Walker, have given many valuable suggestions. Mesdames I. B. Hodgson and E. K. Marks have assisted with many details. Much of this material already has been written (19), and all of it has been collected freely wherever found. There is no intention whatever to say or to imply, "This is it; there is no other way." Rather the hope is to provide assistance for those who want it, and who desire some understanding of the reasons behind the specific directions given.

The illustrations were drawn by Eugene Herrling.

The manuscript was approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

ject for a bite and on that subject for another. Nothing is really accomplished except possibly starting an irritation and a place to scratch.

The results of research are valid only in accord with the reliability of the methods employed and with the accuracy of their interpretation. After an observation has been recorded or an experiment has been made, a strict and seldom-broken rule requires that a report of such work must not be published for the use of others until repeated determinations have been made and before the results have been satisfactorily analyzed. The simpler experiments are commonly made at least in duplicate or triplicate with suitable controls and are carried through three separate times. A good investigator does not become so enthusiastic about an experiment that he fails to view it impartially and to accept sound evidence against it. On the contrary, before publishing he makes every reasonable effort to find an error in the procedure followed, in the experiment itself, or in the conclusions drawn from it.

The reproducible experiment is essential for scientific progress. So the writer should give the reader all information necessary for repeating the work. Likewise, the writer should be reasonably sure that a competent man, when he repeats the work, will find similar data and parallel conclusions.

However, when significant results are actually ready, nothing should delay a prompt and adequate release. Probably as many writers commit sins of omission, which are hard to see, as sins of commission. Scientists in publicly supported institutions have a special responsibility to give promptly to the public the benefit of their verified investigations. The first and authoritative announcement of new research is best made before a scientific society or through publication in a technical magazine. Simultaneous popular releases, when desirable, can be arranged for the press and radio. Such releases must have an entirely different style, which few technical men ever achieve. For such writing a journalist may be secured, providing the scientist corrects his technical errors.

Since different writers achieve satisfactory technical manuscripts by various means, the following suggestions apply only in case there is no better way. Some pains have been taken to explain the philosophy behind certain recommended procedures. These did not descend ready-made from "Minerva" in an armchair; they have developed through many years of toil; they have come from disappointment as well as from satisfaction.

FINE FIGURES MAKE FINE PAPERS

Writing is obviously much easier when one has the subject matter clearly in mind and actually before him. Consequently, many skillful investigators first prepare—at least in temporary form—their pictures, line drawings, graphs, and tables. These items, as well as the text, they condense as much as may be consistent with clarity.

Illustrations Are Never Too Good

The first impression an article makes commonly depends on the character of the illustration. A good piece of writing loses considerable value if the

illustrations are poor. To be sure, one may not be entirely just, but he is realistic to feel that fine figures make fine publications. The best possible text figures are never better than they need to be!

Illustrations are usually photographs or line drawings. As part of the printing procedure, these are made into engravings: the photographs are converted into half tones, the line drawings into zinc etchings.

Scientific journals cannot afford to use illustrations as mere embellishments for a paper. Each illustration must serve a purpose: it should explain a new technique or apparatus; it should amplify a taxonomic description; it should emphasize the appearance of experimental material; or it should summarize data that are too numerous to present in the text.

If he has several photographs, the author should group them together for economy wherever possible. The same applies to line drawings. Photographs and drawings, however, are seldom combined.

Photographs

First, let us consider photographs, which should be taken so that unimportant and distracting items are left out. A glossy finish gives best reproduction (10, 11). Prints should have clear, white backgrounds and strong contrasts, because the half tones usually have less contrast and less detail than the originals.

Photographs are mounted on heavy paperboard, one side of which has a hard, smooth, white surface. Although paste may be employed, wrinkles frequently appear that make the illustrations unacceptable. Rubber mounting cement is commonly used as follows: the back of the print and the smooth, white face of the paperboard are both smeared with a thin layer of the mounting cement. After drying a few minutes, when both appear sticky, the photograph is laid in position and pressed down with a cloth. Any excess of cement on the paperboard or picture is removed by rubbing with a cloth or soft eraser.

When several pictures are mounted together in one figure, each one is lettered, *e.g.*, A, B, C, D, . . . in the lower left-hand corner from left to right and from top to bottom. The waxy surface on all glossy prints is cleaned away from a small area with a touch of ether or an eraser, and the lettering is done with India ink directly on the picture. To do this the author employs either a skillful hand or a commercial lettering guide. If the picture is black, the letter may be outlined with white ink or placed on a small white ink circle. Pasted, cut-out, block letters usually are too heavy to look well and have a poor chance of sticking on the waxy surface.

Because the photographs or drawings must go to an engraver, they are separated from the manuscript and need complete identification. Each illustration desirably carries the following information: (1) the name of the author, (2) the title of the manuscript, (3) the figure number, and (4) the legend, here as well as with the text. These items may be neatly written or, preferably, typed on white paper, then pasted on the paperboard back.

Legends should appear in the text where the first reference is made to the figure. Although it should be brief, it should ordinarily make the illustration clear without reference to the text. Magnifications given for photomicrographs should be calculated according to the size as finally printed.

Finally, whether with a photograph, drawing, or graph, much tender care in preparing good figures can go down the drain unless they are well protected. Innocent paper clips or pencil marks on the previous page can make ruinous dents. Smudges from soiled fingers or carbon paper often show up surprisingly in printing. Protecting the picture with a "flap" of thin paper, pasted first on the paperboard back, then drawn over the face, is decidedly worth while. Likewise, pictures need heavy cardboard protection against creases, folds, and broken corners in the mail. How many writers learn this the hard way!

Drawings over Photographs

When a photograph or a photomicrograph is poor or contains much distracting, unnecessary material, which cannot be trimmed away, the author can make a line drawing on the print (13). Best results come from an enlargement done on paper with a matte surface. The drawing is then made with waterproof black ink directly over the wax-free photograph. After the ink has been thoroughly dried, the silver image of the print is removed in an iodine solution, made by grinding 5 gm. of iodine and 15 gm. of potassium iodide in a little water, until they are dissolved, and by diluting to 500 ml. with water. A few minutes after all traces of the photograph have disappeared, the print is rinsed in water and then the iodine color is bleached for about 5 minutes in 20 per cent "hypo" solution. Because the waterproof ink smears easily, it must not be touched while the paper is wet. The print is washed in water, pinned to a flat, blotting surface, dried in a vertical position, and pressed with a flatiron. Some further drawing often is necessary on the dried print.

Photographs were considered in earlier days to be more convincing than drawings because "photographs don't lie." This naive idea was long ago given up in recognition of photographic skill,² even without retouching. Whichever presents the evidence more clearly is obviously the one to use.

Originals Made to Fit the Page

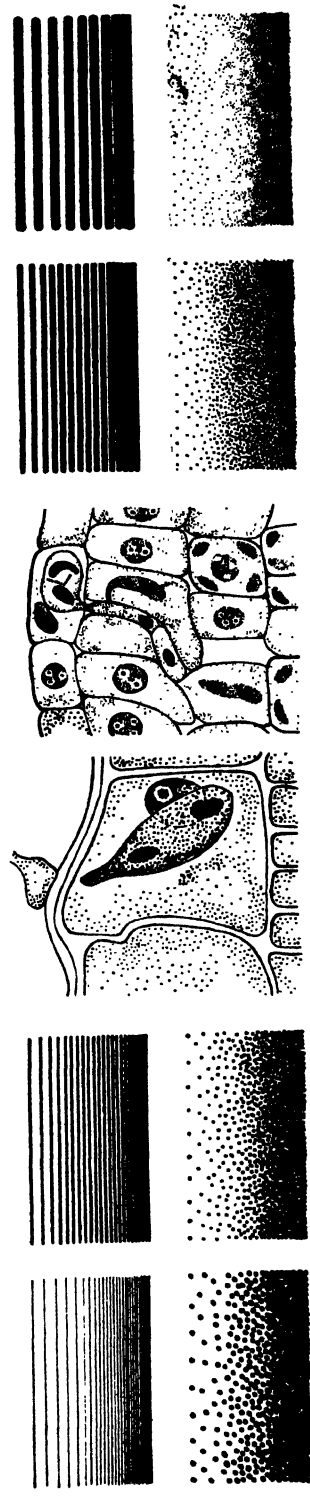
The size of an original illustration, whether photograph, drawing, or graph, requires adjustment in relation to the printed page. An author can frequently trim from photographs much unnecessary surface, such as flower-pots, tops of test tubes, or background, and thus allow more space for the center of interest; or he turns some photographs sideways for an improved arrangement. Graphs and drawings usually come out best when they are made 3 or 4 times as large as the final cut because small imperfections dis-

² Most fishermen know that if they should hold a 12-inch fish at arm's length in front of a yardstick and should photograph it with an ordinary camera, the film would record a fish over 18 inches long.

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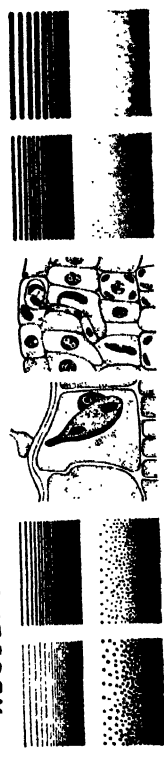
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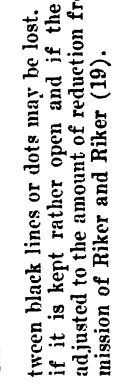


Fig. 1. Letters, lines, and dots in relation to reduction for the printed page. Top, original size. Lower left, reduced to $\frac{1}{2}$. Lower right, reduced to $\frac{1}{4}$. Thin black lines hold up well, but small black dots and small white spaces between black lines or dots may be lost. Delicate shading may be obtained if it is kept rather open and if the size and spacing of the dots are adjusted to the amount of reduction from the original. Reprinted by permission of Riker and Riker (19).

appear with the reduction. The original illustration can be made with dimensions that fit the page³ in the following way: On the bottom of a large sheet of paper the author draws a line on which are marked 1, 2, and 4 times the width of the printed page. At the left end of this base line he draws a vertical line on which he marks the height of the page, less the space the legend will occupy. This he approximates by counting the number of words in the legend and by measuring an already printed legend of similar length. He then marks on the vertical line 1, 2, and 4 times the corrected page height, and he completes the corresponding 3 rectangles. He constructs intermediate rectangles as desired. If the final illustration is to occupy the entire page, $\frac{1}{2}$ of the page, $\frac{1}{3}$ of the page . . . , the original illustration is made to occupy all, $\frac{1}{2}$, $\frac{1}{3}$. . . of whatever rectangle is selected. On the limiting dimension, usually the width, the author marks with blue pencil the size he desires, *e.g.*, "Reduce to $4\frac{5}{8}$ inches."

Balance in Line Drawings and Graphs

Line drawings and graphs are made with India ink, usually on tracing cloth, Bristol board, or the equivalent, and sometimes on celluloid sheets

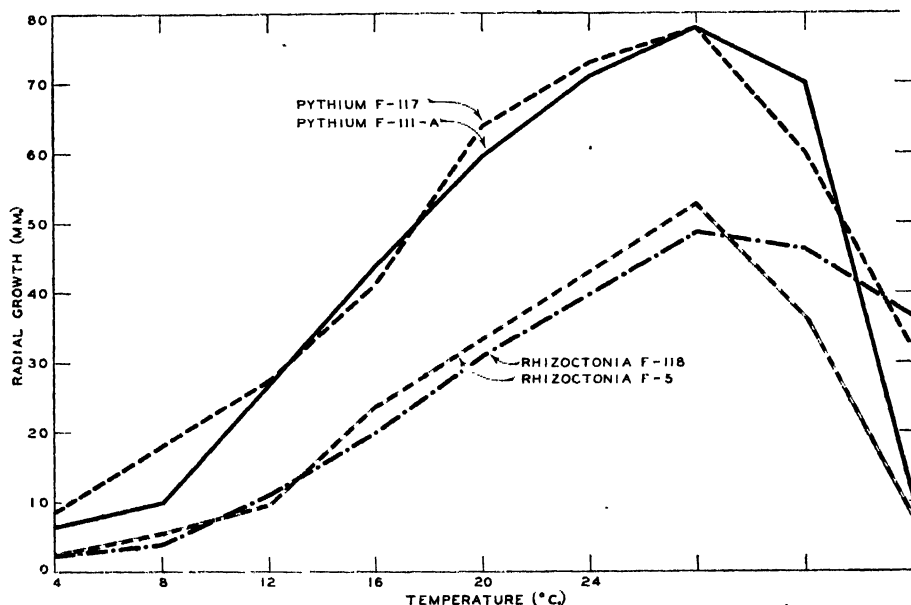


FIG. 2. Temperature and radial growth of four damping-off fungi on potato-dextrose agar in Petri dishes.

(24). The engravers prefer originals rather than photographic copies. The original is essential if a rectangle has been copied askew—and usually it has been.

The size and weight of letters, numbers, lines, and dots used in the origi-

³ For 1946 a page of PHYTOPATHOLOGY measures $4\frac{5}{8}$ by $7\frac{1}{2}$ inches; of the JOURNAL OF AGRICULTURAL RESEARCH, $4\frac{1}{16}$ by $7\frac{1}{8}$ inches; and of the AMERICAN JOURNAL OF BOTANY, $6\frac{1}{8}$ by $8\frac{1}{4}$ inches, with 2 columns 3 inches wide.

nal drawings and graphs require planning, particularly when reduction is contemplated. A guide appears in figure 1.

Graphs and tables are used to present numerical data. Graphs are not only simpler and easier to comprehend than tables, but also they are cheaper to set. Since the style in graphs varies enormously, the beginner may read Anderson's (3) discussion and examine the best graphs in recent publications for acceptable methods.

An example of a relatively simple graph is in figure 2. These data are representative of 3 separate trials; the results of 2 are omitted. Although short, the legend makes the graph clear without reference to the text and with little, if any, repetition of the text. This graph was made with India ink on Bristol board. The size of the original was calculated by measuring the space it should occupy on the printed page and by making the original 4 time as large. The letters and numbers were made with commercial lettering guides but printed letters of the right size may be used. For best appearance the white area between letters needs to be approximately the same in all cases. This means that a C and O must be nearer together than an M and N. The letters and numbers were large enough on the original (for the actual size of the original, make comparisons on figure 1) to read even after reduction, which practically eliminated certain imperfections obvious on the original. Letters smaller than this on the printed page are too hard to read. The spacing of critical points of the scales on the side and bottom is proportioned according to a definite arithmetic (or geometric) progression. Equal spacing between numbers is not acceptable in a series like the following: 2, 4, 10, 16, 20, 50, 100, 150.

There are no vertical and cross lines in the graph proper in this case because a few millimeters or a degree or two are not important, and because the general situation is observed more easily this way. However, the beginnings of such lines do appear for clarity and for anyone who wishes to complete them. A few light guiding lines may be helpful sometimes where the exact reading is critical or if they do not distract from the essential features. The meaning of each graph line is indicated right at the line itself, which is heavier than the scale lines. Four graph lines are easily followed, but more than 5 or 6 become confusing. A continuous graph line between actual points of measurement is justified in this figure because measurements at intermediate points would probably fall near or on the line. However, in a different experiment, if a series of grain varieties or chemicals was placed at the bottom, and percentage of infection at the side, vertical bars instead of continuous lines would be needed because there are no intermediate points. This is discussed in more detail by Anderson (3). He also explains that the preparations of such bars and other conventionalized figures are frequently easier when suitable cross hatching on transparent film (secured from any good art store) is applied properly.

TABLES HAVE MANY POSSIBILITIES

Data too complex or discontinuous for graphs or for text discussion often

appear best in tables. Since their typesetting cost is 2 or 3 times that of ordinary composition, they should be used frugally and only when well organized.

While the acceptable styles vary enormously, an example (Table 1) may be useful. The data are representative, and more similar data have been omitted. The table has been prepared with maximum simplicity. The legend is short, but it is clearly worded, as are the column headings, so that the reader will understand the table without reference to the text. There is little, if any, repetition in the text of data given in the table. The columns have been arranged to fit on the printed page.⁴ Only the headings, proper names, and items in the left column begin with capital letters. Units of quantity (days, number, per cent, etc.) may be set in italics at the top of figure columns. Negative results are indicated by ciphers, absence of data by dots. Only those decimal points appear which are clearly significant.

⁴ The space occupied by letters and numbers can be estimated by the 8 point type in the following lines:

abcdefghijklmnopqrstuvwxyzabcdefghijklmnopqrstuvwxyzabcdefghijklmnopqrstuvwxyz
ABCDEFGHIJKLMNOPQRSTUVWXYZABCDEFGHIJKLMNOPQRSTUVWXYZ
123456789012345678901234567890123456789012345678901234567890123456789

By counting letters, numbers, and at least 1 character for the space between columns, one can estimate the space a table will occupy. The large number of different possible arrangements, discussed in detail by Anderson (3), enables one to arrange almost any material to fit the page. For example, with 3 criteria of classification, A, B, and C, there are 12 possibilities, thus:

A A		B B		A A		B B	
C		C		b b	b b	a a	a a
b		a					
b		a		C		C	
C		C		C		C	
b		a					
b		a					

C C		B B		C C		B B	
A		A		b b	b b	c c	c c
b		c					
b		c		A		A	
A		A		A		A	
b		c					
b		c					

A A		C C		A A		C C	
B		B		c c	c c	a a	a a
c		a					
c		a		B		B	
B		B		B		B	
c		a					
c		a					

Non-significant decimals indicate a lack of discrimination. When they are used, they are uniform in a single column. "Ditto" is indicated not by ditto marks ("") but by Do . . . or . . . do . . . , respectively, in the first or later column. Footnotes often supply information otherwise requiring a column. They are indicated by raised small letters. However, too many footnotes may be confusing. The authors can find in the literature many good examples to follow.

TABLE 1.—Distribution in 1936 of *Pythium* and *Rhizoctonia* on red pine seedlings in Wisconsin forest nurseries

Location	Soil		Seedlings plated	Platings positive ^b	Yield of positive platings	
	Type	Reac- tion ^a			<i>Pythium</i>	<i>Rhizoctonia</i>
		pH	Number	Number	Per cent	Per cent
Rhinelanders	Vilas sand	5.0 ^c	8	1	0	100
Port Edwards	Plainfield sand	5.0 ^c	56	54	0	100
Do	do	5.1 ^c	82	81	19	81
Gordon	do	5.2 ^c	13	7	0	100
Madison	silt loam	5.3	17	15	20	80
Trout Lake	Vilas sand ^d	.	19	10	80	20

^a Determinations were made with a quinhydrone electrode and checked with a glass electrode.
^b Positive plates yielded either *Pythium* or *Rhizoctonia*; all other plates were negative or yielded saprophytes or dubious parasites.
^c Treated at seeding with 2 per cent sulphuric acid to control damping-off.
^d Instead of soil from a nursery, this was soil from a nearby woods.

Particularly in a summary table when statistical analysis has been employed, one should mention the procedure and give an indication of the variability in the results; for example, the minimum significant difference or a summary of the analysis of variance.

Editors usually will take details of some representative data which are necessary to explain the procedure. After this they accept only summarized or well-digested data which give the results. Obviously, an author cannot publish all the data that have accumulated.

The tentative completion of tables, text figures, and, in addition, the pertinent literature citations before planning the details of the manuscript frequently saves considerable time in revision.

THE OUTLINE MARKS THE WAY

The preparation of a manuscript, just as with the itinerary for a trip, begins with a detailed plan, which frequently has been marked out already in the research program. The kinds of plans vary, but they should indicate the most suitable way, like road markers, and avoid meandering lanes. Most writers, unless they have something better, follow a conventional outline such as the following:

Title. This desirably is concise, descriptive, and favorable to accurate

indexing.⁵ Thus another investigator is more likely to find it in his search of the literature. Further discussion is given by Oberly (14).

Introduction. This part often contains (1) a statement of the general subject, (2) the orientation, setting, and foundation on which the present investigation was made, but not a general literature review, and (3) the purpose of the studies described.

Materials and methods. This section usually explains broader aspects of what was used and how the work was done. However, certain details about individual experiments may fit in better in the specific section where they are described. The validity of research has its foundation on the method followed by the investigator. The validity of his technique and the logic of his interpretations need to be clearly apparent and must be acceptable (cf. Rawlins, 17; Riker and Riker, 19).

Observations, experiments, and results. This is the main part of the manuscript, containing brief descriptions of observations and experiments. Representative data and summaries of results should appear in a clear, concise, and logical form. Unimportant experiments and results, details of duplications, or data that cannot be interpreted are left out. Various subheadings may be useful. Under each the data may be presented in their logical order and without regard to the sequence in which the experiments were performed.

Discussion. In this portion the writer may answer the question "So what?" as he interprets the various data in relation to one another and to the foundation given in the "Introduction." He clarifies the meanings and implications of the various results and may indicate possible future developments. The reasoning done must be accurate and in accord with a recognized method of logic. Although in this place he can take a panoramic rather than a keyhole view of the situation, he wisely avoids wringing out of the results the last drop of theoretical implication. Summarization is avoided. If there is nothing important to discuss, this section should be omitted.

Summary. This is a brief résumé of the results together with the important conclusions, and not a general statement of the field of study. In a "note" occupying less than two printed pages, the last paragraph serves as a summary.

Literature cited. Five or more references are placed in this separate section. Four or less appear as footnotes.

Such an outline, and particularly "Observations, Experiments and Results," may be elaborated until every separate item of the paper has an entry. If the author does this in detail, he saves much time in later reor-

⁵ For example, "A new oak disease" is inadequate. "The general importance, differential symptoms, and causal fungus responsible for a new wilt disease severe on red and black oaks" is too long. "The significance, symptoms, and cause of oak wilt" covers the essential points. "Studies on . . ." is omitted because it is implied before almost every title. The Latin name of the fungus is usually omitted, especially if its common name or the name of the disease appears. If the Latin name is used, the authority is omitted in the title, but is given in the text where the name first appears.

ganization, since he can hold in mind the divisions of the subject and their relations to one another more easily during the outline stage. The actual writing of the paper moves along better because final units in the outline furnish the subjects for the paragraphs. Some writers develop the outline in such a way as to provide not only the topics but also the topic sentences of the paragraphs. At first, this procedure may result in mechanical writing, but, in time, this is easily overcome. Even so, it is better than disorganization.

PREPARATION OF THE TEXT

Writing a manuscript need not be the disagreeable task often made of it. There is a technique for writing just as there is for making single-spore isolations—except that writing takes more time and patience to learn. However, once the skill is acquired, it can be a delightful means of self-expression.

A Writer Has Responsibility

An investigator stands before his colleagues and the public as an educated man and has the obligation to show it in his writing. He makes correct use of grammar, rhetoric, and logic; he keeps his readers in mind; and he shows them the consideration and courtesy of simple and clear exposition. He does not string together ponderous phrases without continuity, like wash on a back-alley line.

The aim of publication is to increase knowledge (*e.g.*, interpret the past, take care of the present, and provide for the future) and to leave the field clearer than it was. The last calls for the proper balance throughout of unity, coherence, emphasis, and accuracy. It is necessary to use language that not only gives the information but also gives it so clearly that it cannot be misunderstood. "Every paragraph and every sentence in your paper should receive careful and repeated consideration, first, as to whether it tells the exact truth; second, as to whether it is absolutely clear, *i.e.*, will convey the same meaning to all as to yourself (try it on your friends, if they will submit to it); third, as to whether it is complete, or requires various additions or qualifications—science is an eternal qualification; fourth, as to whether the sentences in it are entirely logical and move convincingly toward your final conclusions. These things can be determined only by repeated readings and much pondering" (21).

A publication is not judged by its length but (1) by the message it carries, (2) by the amount of well-directed and well-digested work it represents, (3) by the discrimination shown in distinguishing between the important and the relatively unimportant, and (4) by the conciseness in presenting the essential features. As a rule the more definitely a fact has been established by an investigator the more directly and simply it can be presented. The doubtful facts have to be hedged about with explanations, qualifications, and cautions. So brevity is an important quality.

A Premium on Brevity

Many writers use roundabout, wordy expressions^a and include much unnecessary detail. Then they are puzzled when reviewers say, "Condense the manuscript to one-half its present volume." Such writers may think they are asked to omit many of their experiments and may feel resentment and frustration.

Among the diseases of manuscripts, a diarrhea of words is perhaps the most common. A representative case may clarify the symptoms and cure. Do you think the following paragraph, about the propagation of white pine selected for resistance to blister rust, is exaggerated? If so, you should see some examples collected from papers actually submitted.

"The multiplication of resistant white pine trees by means of making grafts has been already demonstrated as a feasible possibility. On an experimental basis, as was explained earlier, over 1000 grafts have been made and grown successfully during these studies in both green house and field conditions. Yet a grafted tree has been found relatively expensive because of the necessity, according to present technique, of using green house space in the winter time and cold frames in the spring. Counting scions, stock, pots, labor, *et cetera*, each graft is estimated to cost about 12 cents. This cost might be reduced somewhat if further research will be done to further improve the methods employed. It might also be reduced by putting this operation on the basis of a large scale commercial production. Even at this relatively high figure grafting may now be done as a practical possibility due to the high price paid for certain types of ornamental planting. But the most promising means that has appeared of reducing the cost of propagating rust resistant white pine trees is through the rooting of cuttings." More things than cuttle fish hide themselves in their own ink (cf. Maverick, 12; Barzun, 5).

This paragraph can be reduced to about one-third the space by omitting unimportant detail, can be clarified, and can be corrected (*e.g.*, it contains a dangling participle, some awkward phrases and four misspelled words) as follows (cf. Riker *et al.*, 20):

"Grafting white pine is feasible for experiments. Yet grafted trees are relatively expensive now (about 12 cents each) because they are propagated

^a For example, some expressions with their shorter equivalents follow:

it will be seen from the foregoing figures = these figures show

it would thus appear that = seemingly or apparently

it is true that = admittedly

it is this that = this

the fact that the cultures grew shows = the growth shows

the question as to whether = whether

during the time that = while

at an earlier date = previously

with reference to = about or concerning

the treatment having been performed = after treatment

under greenhouse conditions = in the greenhouse

goes under the name of = is called

plants exhibited good growth = plants grew well

conducted inoculation experiments on = inoculated

in a greenhouse. Even so, grafting is possible for certain types of ornamental planting. Although improved technique and large-scale commercial production might reduce this cost, it will doubtless remain higher than that for cuttings."

Such condensation is prized by scientists who seek as many facts and ideas as possible in ten minutes of reading—but it may be anathema both to journalists writing at so much per word and to readers seeking effortless entertainment.

The rearrangement of words and phrases is really an interesting game. Of course, manuscripts can be cryptic and lose precision if condensed too much. While many adolescents might disagree, it would be a shame to reduce Caesar's "Commentaries" to Sherman's "War is hell." The beginner might try condensing manuscripts written by others⁷ until he discovers how much fun it is and how to do it well. It is much more difficult for him to see and to correct the weakness in his own composition.

Being Dull or Interesting

In the choice and arrangement of words lies the difference between dull and interesting composition—providing one has something to say. Being dull involves, for example, (1) numerous clichés and platitudes, (2) verbosity and circumlocution (For goodness sake, come to the point!), (3) obscurity (What does he mean, anyway?), (4) the excessive use of the passive voice, (5) long pretentious words in stilted phrases,⁸ and (6) expressions that do not fit together. All of these can be overcome with a little care. While perhaps no one would introduce into a report on potato late blight the lure, suspense, and drama of love and murder, still technical writing need not make us yawn. For example, one can at least aim at satisfying organization, directness, brevity, lucidity, concreteness, and even an occasional daring or sinewy phrase. Words and expressions that suggest pictures and actions help to hold interest and to clarify meaning. Overstreet (15) has developed further this important subject.⁹

Accuracy—a Prime Necessity

The definitions of words are frequent sources of trouble (cf. Committee, 8, 9). Words are the most valuable of scientific tools; they convey facts and ideas to others; but they can be dangerous. Occasionally some one extends a meaning without bothering to see whether it is valid, and thus he has a false sense of security. Many an argument has developed over different concepts for the same word. For example, "single-cell culture" applied to bacteria means for one person a culture from a single bacterial colony in a Petri dish (and thus no direct evidence that it is free from mixture), while

⁷ If you do try it, please use discretion. It may be no safer to criticize a man's brain child than to slap his little Tommie or Susie.

⁸ They remind Maverick (12) of the ridiculous strutting pomposity and the "gobbledygook" of his turkey gobbler.

⁹ We have just seen a new and excellent aid to writing for easy reading. It is by Rudolph Flesch, "The art of plain talk," 210 pp., New York [1946].

for another it means a culture derived from an observed and a mechanically picked individual bacterium. If there is danger of a critical word being misinterpreted, the author may wisely explain just what he does mean.

Emphatic expressions about conclusions are undesirable because of the space they occupy and the suspicions they arouse. The clearly stated fact needs no reenforcing. If a writer says, "The data clearly show beyond possible question that . . .," it suggests that he needs to convince himself. It reminds the reader of the timid boy who whistled to keep up his courage.

Teleological statements always arouse criticism. Two sentences may serve as examples: "The stomata ~~are~~ closed in order to reduce further water loss from the leaves." "The germ tubes formed appressoria on the cuticle to help send infection pegs into the tissue." These expressions, which suggest that stomata and germ tubes had a purpose for what they did, indicate illogical thinking, careless composition, and especially departure from the approved scientific attitude.

Trade names of fungicides are far from precise. They have appeared on materials that have been changed again and again in composition. Even when the container gives the formula, another investigator, especially in future years, may not know exactly what it was. Such trade names should not be used unless the chemistry of the product is given. When used they are capitalized (Semesan, Vigoro, Hudson sprayer . . .).

Accuracy is a critical requirement in technical writing. The scientist must scatter the fog of abstract or obscure expressions and let the light shine clearly on the concrete base of reality. If he is careless in reporting his work, he may be suspected of being careless in his experiments, in taking data, and even in thinking about them. The design and interpretation of the experiments need to be clearly in accord with logical scientific methods (cf. Wolf, 27). Lucidity is the sovereign politeness of the writer; he must make sure that his words can be given no unintended other meaning.

To read accurately seems to be among the hardest things in this world. To misunderstand is among the easiest—because we tend to get the idea we expect to find, or we wish to find; and too often we may twist the meaning to make it what we like (cf. Barzun, 5). Poor writing makes hard reading. Repeated lack of precision through laziness or scatterbrains suggests the adulteration of food; there is possible danger and certainly bad taste.

A clear and sharp line needs to be drawn between fact and opinion, between reason and wishful thinking. The scientist should be objective and should formulate only conclusions justified by the evidence. Deductions from preconceived notions or, much worse, from prejudice, indicate a mind closed to impartial and honest reasoning. Sooner or later they detract from a man's standing and from scientific progress. Administrative pressure or personal ambition may lead to the misinterpretation or over-enthusiastic reporting of a valid new discovery. Examples are common. The salvation of the plant world has been announced repeatedly by extravagant and unjustified claims for such valuable items as fertilizers, sprays, seed treatments,

hormones, and a long list of others. When an investigator extends a generalization, he is probably also extending his neck. He may well recall that "no generalization is true—not even this one."

One needs to be objective and to show consideration for his colleagues, especially when they are in error. Comments about one's own research and that of others are in bad taste when personal, and in good form when factual. Disparaging statements commonly boomerang to the disadvantage of the writer. While spectators may enjoy the free show, who can throw mud and still keep his hands clean? Although Erwin F. Smith practically macerated Fisher in their well-known controversy, Smith lived to be ashamed of the way he did it. Difference of opinion and good-natured controversy are healthful and stimulating, but "dog fights" are bad. Most conflicts can be settled best through correspondence, in conference, or by further experiments without wasting space in print. After all, the question is not, "Who is right?" but "What is true?"

This objective approach, when applied to earlier contributions by others, allots to each the treatment it deserves in relation both to the present contribution and to the subject as a whole. A nationalistic or a personal twist of the credit assigned to earlier work, either by omission or by minimizing its significance, can only corrode the writer's reputation. The white radiance of established fact shines more beautifully if it is unsullied either by the sweat and blood of acrimonious controversy or by the odor of piffing jealousy and unenlightened ambition.

Bad and Good Beginnings

Novices in technical writing frequently get poor results for several reasons: (1) They have trouble writing a good "Introduction." This is one of the most difficult parts of a paper. Even when the statement of the problem, the scope of the work, the background, and the purposes are clear from the outline, beginners often have trouble. If they do, they may well pass over it lightly or even skip to "Materials and Methods,"—perhaps the easiest part for the initial effort. (2) They have not finished, at least in preliminary form, the text figures or tables and have insufficiently digested their results. They begin to write before they have decided which results should be left out, which used, and what they mean. (3) They may be discouraged because in the first draft they seek perfection; therefore, they may wreck the train of thought on the spelling of "desiccate," a dangling participle, or the choice between 50 cc. and 50 ml. (4) They allow insufficient time for writing. When there is a deadline date, the pressure increases the difficulty. For example, after reviewing the literature and after completing and digesting the experiments, a graduate student should allow 3 entire months for writing his doctor's thesis. Even experienced and skillful writers seldom produce an excellent manuscript without going over it many times, without having several clean copies made, and without laying it aside to cool before final revision.

A happy way to begin is to recognize that for the first draft the all important thing is to transfer the thoughts to paper. With an outline to serve as a guide down the main line and away from sidetracks, one can run light-heartedly and with disregard for spelling, punctuation, and rhetoric which might wreck the train of thought. At this stage the self-expression of personality appears which provides style and raises an otherwise dull subject to the level of pleasant reading. So long as the "spirit moves," no question of detail is permitted to interfere. Some people find it helpful to imagine several listeners and to write as if talking to them. They may include everything possibly significant and later eliminate nonessentials during revisions of separate parts. It is easier to cross off than to make inserts.

Paragraphs Are Single Units

Many writers place a single paragraph on one sheet of paper to avoid the clutter of cutting and pasting during later revisions. Then they can subtract, add, or move a single unit without disturbing other sections.

The composition of a paragraph varies with different circumstances, but desirably contains only one central idea. An investigator may consider a structure something like this: (1) topic, in which the subject, but not the conclusion, is stated; (2) explanation of topic, in which appear any necessary clarifications of terms or greater descriptions of various items; (3) central part, in which is given the detail regarding the topic discussed; (4) conclusions, in which the writer's estimate of the meaning of this detail is explained; and (5) transition, in which the connection between this paragraph and the one following is shown. The transition may appear as a word or phrase in the first sentence of the next paragraph.

The chief objection to this form is a tendency to be stilted unless the transitions are skillfully handled, but this is a much smaller fault than obscurity. The topic sentences sometimes cause difficulty because, instead of the topic, writers place the conclusion first; then they endeavor to justify it by the material that follows. This suggests an effort to prove a preconceived idea rather than to make an impartial evaluation of the evidence. Herein lies one of the basic differences between technical and news writing: a reporter tells the important thing first; he gives various details later.

The chief advantage of writing so that the topic is clearly stated in the first sentence (and better still in the first phrase of the first sentence) is that the reader can glance rapidly over the article; he can locate easily the paragraphs in which he is interested: he can skip everything else. And this is, after all, a critical test for good writing in a technical paper. This system is a little hard at first. But once a writer develops the habit, he saves much time in hunting through the manuscript for a particular item, especially after a clean copy has been made. Likewise, readers can glance rapidly through a stack of papers written in this way. Articles not so prepared they may file away for reading in a leisurely time—which never comes.

Admittedly, there are other approaches. However, technical writing

has certain limitations. They prevent the writer from employing some of the interesting techniques useful in newspapers and popular magazines. For example, (1) a scientist must use the technical jargon (the shorthand) of his subject. It is required for brevity, clarity, and precision, even though it may be unintelligible to those untrained in this subject. (2) He needs to be brief. (3) He should avoid even the appearance of personal bias and should state first the facts and then draw the conclusions. (4) When he must walk in those fields where emotions blossom to influence judgment, he considers only the valid data and logical inferences from them. He shuns the assessment of values he cannot measure. He differentiates fact from opinion. Such conditions are rather incompatible with the "human interest" sought by journalists. So within rather exacting requirements each scientist will use the best way for his purpose. But he should not hope to find a "short cut" for painstaking revisions.

Revision Requires Time and Care

Once the first draft has been made, its revision and correction call for a different approach. A refresher glance at one of the many good handbooks of composition (*e.g.*, Woolley, Scott, and Berdahl, 28) is sure to be helpful.

The correction of the text involves many details which are frequently merely conventions adopted for convenience, uniformity, and precision. Terminology; spelling including capitalization, abbreviation, and compounding words; punctuation; and citations are a few of the questions requiring special attention. Except where otherwise stated, PHYTOPATHOLOGY aims to follow the Public Printer (16).¹⁰ Attention is directed particularly to such abbreviations as: cc. for cubic centimeter (space); ml. for milliliter (volume, instead of cc.); gm. for gram; and µg. for microgram (instead of gamma)—all with periods. For spelling and common meanings of words, PHYTOPATHOLOGY follows Webster's New International Dictionary; for special definitions,¹¹ recent reports (8, 9); for common names of plants, Standardized Plant Names (2); and for scientific names, the International Rules of Botanical Nomenclature (7). However, dissenters have their rights, and most editors defer to authors with valid arguments. The description of a new fungus species should have a summary in Latin, and that of a new bacterial species, in English. Among others, Trelease and Yule (22) discuss the preparation of scientific and technical papers, and Woglum (26) has assembled a list of improperly used words.

An alteration in one place frequently calls for a survey of the text for

¹⁰ For sale by the Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C.

¹¹ Some technical words are hard to define. The committee reports commonly give the best definitions, but even they are more or less tentative. For example, among the various definitions for "plant diseases" we have this facetious one, "Plant diseases are the things plant pathologists work on." If you know what we mean, a definition is not necessary; if you do not, it does not help much. Are we reduced to saying, "Plant disease—you know what I mean"?

some distance, before and after the change, for corrections dependent on the first. This deserves special emphasis.

Should such details be left for the Editorial Board? Not until the Society pays its editors. Meantime, they perform an enormous and thankless "labor of love," and they deserve to receive manuscripts in good form.

The criticism of a manuscript by well-informed colleagues is eminently desirable. Even though it has been prepared with extreme care, has been reread critically by the writer a dozen or more times, and has received a number of retypings, the suggestions of colleagues are sure to reveal various rough places and to discover statements which might be construed in two ways. It is a common experience that a man cannot edit his own manuscript. The examination by others will guard against a misinterpretation of the data presented, against drawing conclusions from inadequate data, and against publishing a paper that may later be embarrassing, not only to the author but also to others in the same institution. Tabular and statistical matter particularly are to be checked. The values of such a procedure so far outweigh the extra labor involved that such a review is required by most editors.

Those Troublesome References

References to other publications present many opportunities for error. Just as editors frown on long literature reviews, so they discourage an excessive number of citations. Unless they are really necessary, a large number makes a paper appear pedantic rather than learned. How often we see a general statement followed by a string of citations when one or two would serve as examples.

An easy method, while preparing a manuscript, is to enter in the text the name of the author with the year of publication. If the same author has several publications in a year, they are designated by letters (*e.g.*, Jones, 1935a, 1935b, 1935c. . .).

The complete citation may be placed on a card of convenient size, the reference cards being kept in alphabetical order. The form of the citation is a convention and follows the best form in recent issues. Examples appear at the end of this paper. In case of doubt, the plan suggested by Whitlock (25) is excellent. The abbreviations for the titles of the periodicals may be prepared and interpreted according to Bartholow's (4) list. Before the literature cited is typed double spaced from the alphabetically arranged cards, every letter and number of the citation should be checked against the original. Do you think this is a tedious, time-consuming job? Obviously. Should it be left to the editors? Certainly not.

Before the final copy is typed, frequently the author's name and always the year of publication are replaced by numbers,¹² corresponding to those in the alphabetically arranged "Literature Cited," providing there are 5 or more. Fewer citations are handled as footnotes. Once the list is done, any

¹² There are important arguments in favor of leaving the names of the investigators and the year of publication in the text (*cf.* American Journal of Botany).

deletion or addition of a reference means changing many numbers both in the text, where they are easily missed, and in the "Literature Cited." For example, if a reference to a paper by Brown is added or removed, all the numbers applying to papers by authors following Brown in the alphabet must also be changed.

THE FINAL COPY

The final copy is typed double spaced with ample margins on good white bond paper (preferably about 8½ by 11 inches) and with at least 2 carbon copies. Footnotes and legends for text figures and plates are typed immediately below the first notation to them and are set off from the text by a line above and another below. The printer likes a separate page carrying the legends of the text figures. Each table is typed on a separate sheet (so it can be taken out and given to a different typesetter) and inserted as a numbered page in the manuscript immediately after the page on which the table is first mentioned. The manuscript is accompanied by a table of contents showing the rank of the various center headings and a statement of the number of pages, tables, and text figures.

The carbon copies smear less and are more easily read if each page is heated momentarily above the melting point of the wax in the carbon paper. As this wax melts, it is absorbed by the paper. The wax impression smears easily while hot, but is fixed after cooling.

When this final typing and its carbon copies have been made and checked, the author still has several details to consider.

Before a manuscript is sent off for publication, it is hoped that the writer can answer "Yes" to the following questions: (1) Has every reasonable effort been made to find a flaw in the technique used and to discover any errors either in the observations and experiments themselves or in the conclusions drawn from them? (2) Is it reasonably certain that, when able investigators repeat the work, they must secure the same results and draw the same conclusions? (3) Has the size of the manuscript been reduced to the minimum compatible with clarity of presentation? (4) Have at least two qualified colleagues read and criticized the manuscript? (5) Has a competent statistician examined the tables and computations? (6) Do the illustrative materials, tables, and legends meet the requirements of PHYTOPATHOLOGY? Will each figure and table fit well on the printed page? (7) Is there a reference in the text to every table, to every figure, and also to every publication referred to in "Literature Cited?" (8) Has every entry in "Literature Cited" been checked against the original, and is the form of reference suitable for PHYTOPATHOLOGY? (9) Have you prepared a table of contents showing the relative rank of the different headings? Have you written on the first page the total number of pages, text figures, and tables? (10) When the paper was finished, was it laid aside for at least a month and then reexamined critically with a fresh viewpoint and without bias? (11) Has it been approved by the proper administrative office (if

this is required)? (12) Is PHYTOPATHOLOGY the best journal for this manuscript?

INCIDENTAL MATTERS

After a manuscript has been received by the editor, two anonymous but well-qualified referees examine it. The time is shortened when the author sends in a complete duplicate of his paper, except for complex illustrations. While reviewers do their best and are almost always helpful, they are human. The author must make sure that they have not introduced any mistakes. This editorial procedure seems necessary not only to maintain a satisfactory standard but also to assist the authors, so far as is humanly possible, to avoid serious errors.

For any important corrections suggested, the manuscript goes back to the writer. However, when it has only minor editorial changes, the manuscript goes to the printer and the author checks the changes when he reads the galley proof.

While no one needs to achieve perfection, he should meet at least a satisfactory standard. If he fails in this, his paper either will be delayed until he does accomplish it or will be rejected.

After galley proofs are received, errors are indicated by standard proof-reading marks (cf. Riker and Riker, 19; or Public Printer, 16). At this stage only essential alterations are permissible. Any minor changes in wording or punctuation are avoided. These, of course, should have been made earlier on the manuscript, when a few strokes with a pen would have accomplished the results.

Typesetter's errors should be marked in red ink only on the galley proof sheets. The original manuscript should never be altered when read against the galley proof. Author's alterations from copy should be indicated in black ink only on the proof sheets. The printer charges for the latter at the prevailing rate. The change of a single long word may require that the rest of the paragraph be reset.

Abstracts of papers for transmission to Biological Abstracts are requested at the time proof is returned to expedite their publication. Ordinarily, the abstract does not exceed in length 3 per cent of the original paper. A guide for preparing such abstracts is available (6).

Reprints are ordered at the time proof is returned.

SUMMARIZED RECOMMENDATIONS

As you prepare manuscripts for PHYTOPATHOLOGY, you are requested, as far as it is feasible, to follow these recommendations. If at first some of them resemble armchair flats, please consider the explanation for most of them in the preceding pages.

Illustrations

1. Plan in relation to page dimensions and allow for the space needed for a legend.

2. Avoid duplication of illustrative material and an excessive amount. The author may be charged for illustrations and tables which combined occupy more than 20 per cent of the page space.
3. When there are several similar illustrations, combine them into one figure whenever possible, but do not combine photographs and line drawings.
4. Letter units of a composite illustration (A, B, C, . . .) on the picture itself. Letter specific parts of a unit (a, b, c, . . .). The author should employ either a skillful hand, a lettering guide, or suitable type. Do not leave this to the editor.
5. Submit only photographs that are glossy, have plenty of contrast, and have been mounted without wrinkles with rubber cement.
6. Trim away unnecessary parts of photographs (flower pots, backgrounds, operators, nonessential plant parts . . .).
7. Use undiluted India ink drawings or silvertones rather than poor photographs.
8. Prepare drawings for zinc etchings on Bristol board or the equivalent.
9. For graphs avoid green-line graph paper and typewritten labels or symbols. Use undiluted India ink on special blue-line paper, white paperboard, or tracing cloth.
10. Make sure of a heavy, even, steady line; of size, clearness, and openness of lettering or stippling; and of accuracy of scales—all in relation to the reduction planned.
11. If magnifications are indicated, calculate them for reduction of illustration when necessary.
12. Indicate with blue pencil the reduction desired.
13. Prepare legends that are brief but clear with little, if any, repetition of the text. Place author's name, title of manuscript, figure number, and legend on the back of the figure.
14. Pack illustrations well for mailing. Use plenty of corrugated paperboard or the equivalent to avoid wrinkles, folds, cracks, and dents from paper clips.

Tables

1. Avoid long, complex, or undigested tables.
2. Avoid too many tables—the most expensive part of a manuscript to print. The author may be charged for more than 20 per cent all told of illustrative and tabular material.
3. Use the text rather than small tables whenever possible.
4. Arrange the table to fit upright on the page (4½ by 7½ inches in 1946) whenever possible.
5. Number tables with arabic numerals (1, 2, 3, . . .).
6. Provide a complete table heading for every table.
7. Provide clear and concise column headings.
8. Letter footnotes a, b, c,
9. Explain any symbols used in a table.

Text

1. Allow plenty of time for writing. Use a simple, direct style which is condensed, but not so condensed as to be cryptic or to sacrifice precision and clarity.
2. Organize the material following logical sequences and not according to the order in which experiments were performed.
3. Arrange the paper so that a reader can glance over it rapidly and can locate easily any special part he wishes.
4. Revise the manuscript until it has unity, coherence, emphasis, and accuracy, and until it is so clear it cannot be misunderstood.
5. Avoid pompous, pedantic, or wordy expressions and unnecessary detail. However, give all the facts necessary for a well-trained person to repeat the experiments.
6. Be objective regarding your own results and those of others. Avoid teleological statements.
7. Make sure that the reasoning follows a well-recognized system of logic. Likewise, be sure that in each experiment or test you used an acceptable method.
8. Avoid long literature reviews and an excessive number of citations, but do not ignore relevant work of others.
9. Distinguish clearly between statements supported by experimental evidence and those based on speculations.
10. Use headings and sub-heads, as well as good paragraphs, to enable the reader to "skim" the article for its general subject matter and to locate quickly any detailed part he seeks.
11. Provide a summary, usually not longer than 3 per cent of the paper, in which the contributions are stated clearly and succinctly in specific terms rather than in general statements of the field covered.
12. Let tabular data and illustrations speak for themselves. Confine the text discussion to the meaning of the data.
13. Avoid presenting data in duplicate or triplicate, *c.g.*, in graphs and tables, or in graphs, tables, and the text. Choose the best of the three forms and omit the others.
14. Although experiments must be done over and over to make sure of the results, present only representative or summarized data.
15. Explain new terms and terms not in general use. State the chemical composition of fungicides.
16. Provide a Latin summary with the description of a new fungus species.
17. Number tables, text figures, and footnotes (beginning each series with 1, 2, 3, . . .) consecutively throughout the paper.
18. Employ the conventions regarding, for example, abbreviations, plant names, and references to figures, tables, and literature citations.
19. Type the final copy with heavy black ribbon, with double space, and with ample margins on white bond paper. Approximately 8½ by 11 inches is the preferred size for paper. If the first carbon is sent with

the original, more rapid review may be achieved. Keep a carbon both for reference and insurance against loss.

20. Plan papers that will occupy less than 2 printed pages as "Phytopathological Notes."

Literature Citations

1. Handle 4 or less literature citations as footnotes.
2. Arrange 5 or more literature citations alphabetically according to author at the end of the paper under the heading "Literature Cited."
3. Provide a complete citation—author or authors, complete title, complete reference to journal, bulletin, circular, book. . . . For a journal give volume, limiting pages, and year of publication. For a book give edition, total number of pages, year of publication, and city where published.
4. In the body of the paper refer to literature citations by number. See that the numbers in the text and in the "Literature Cited" correspond without any left over.
5. Check every letter and every number in the literature citations against the original.

Finishing Items

1. It is hoped the author can answer "Yes" to the following questions:
 - (a) Have you critically reexamined the methods, experiments, and conclusions?
 - (b) Will further repetitions of the work yield the same results and conclusions?
 - (c) Have you eliminated all unnecessary materials from text, tables, and figures?
 - (d) Have two qualified colleagues criticized the manuscript?
 - (e) Has a competent statistician examined the tables and calculations?
 - (f) Have you met the conventional requirements regarding illustrations, tables, legends, and references to them? Will the text figures and tables fit properly on the printed page?
 - (g) Is there a reference in the text to every table, figure, and item in the "Literature Cited"?
 - (h) Have you checked every entry in the "Literature Cited" against the original?
 - (i) Have you prepared a table of contents showing the ranks of various headings and sub-headings?
 - (j) After the paper was finished, did you lay it aside for a month and reexamine it critically with a fresh viewpoint and without bias?
 - (k) If required, has the proper administrative officer approved it?
 - (l) Is PHYTOPATHOLOGY the best avenue for publication?
2. After two anonymous reviewers have criticized the manuscript, it will be returned to the author, if necessary, for his further consideration.

3. The author may be charged for an excessive number of tables, line cuts, and half tones if such materials all together exceed one-fifth of the total number of pages.
4. Authors will make corrections promptly on the galley proof (not on the original manuscript). Mark typesetter's errors in red ink and author's changes from copy in black ink. Return the proof promptly and send back with it two copies of an abstract and any order for reprints.
5. The author will be charged for any excessive changes of proof from the text.
6. Care in observing these conventions and suggestions will help the editorial staff and will enable colleagues to share the pages available in the journal.

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PHYTOPATHOLOGICAL NOTES

✓
*A Pycnidial Strain of *Macrophomina phaseoli*.¹*—During the course of work on root rots of snap beans in Georgia, a strain of *Macrophomina phaseoli* (Maubl.) Ashby which produces pycnidia and pycnosporos abundantly on agar media was isolated. This pycnidial strain (culture No. 75) was obtained from infected hypocotyl tissue of a snap bean seedling having symptoms of charcoal rot at Experiment, Georgia, on May 25, 1944. The isolate was typical of *M. phaseoli*, forming a black crust of sclerotia over the surface of the medium. In about two weeks pycnidia appeared among the sclerotia. Pycnosporos embedded in a viscous fluid oozed from the pycnidia and collected in white, opaque droplets plainly visible to the naked eye. Fifty pycnosporos taken from a culture on three per cent malt agar measured $14.0-30.8 \times 7.0-11.2 \mu$. A year later 20 single pycnosporos isolations were made from this strain. All of these isolates were similar to the original strain; and all produced pycnosporos on agar media, indicating that the strain was genetically stable.

Among the 58 isolates of *Macrophomina phaseoli* which have been examined in this study, culture No. 75 is the only one that has produced pycnidia on agar media. Attempts were made, therefore, to find some means of inducing the other isolates to form pycnidia in culture. The most satisfactory method tried was the following: Dried snap bean stems were cut in 10-inch lengths and soaked thoroughly in water. The stems were then inserted in large culture tubes 12 inches long, their basal portions being immersed in water to a depth of two inches. After sterilization, the bean stems were inoculated with various isolates of the fungus. The majority of the isolates formed scattered pycnidia somewhere along the bean stems within one month. The pycnidia were often elongated, compound, coralloid structures projecting above the crust of sclerotia. By this method, 35 different isolates of *M. phaseoli* were tested for the production of pycnidia. Of these isolates, 17 were from Georgia and 18 were from various other states. Three of the isolates from Georgia were furnished by Dr. J. L. Weimer, one from Georgia by Dr. G. E. Thompson, four from California by Dr. W. C. Snyder, one from Maryland by Dr. R. W. Leukel, two from Nebraska by Dr. J. E. Livingston, and one from California, four from Texas, and six from Oklahoma by Dr. E. C. Tullis. As is shown in table 1, only 20 of these isolates formed pycnidia. The pycnosporos dimensions recorded in the table were obtained from measurements of 25 pycnosporos from each culture. The isolates from sorghum appeared to have been maintained in culture for some time and to have degenerated to forms that produced an abundance of light-colored aerial mycelium and few sclerotia. Changes in the fungus produced by growth on artificial media may explain the failure of these isolates to produce pycnidia. Most isolates of *M. phaseoli* undergo such changes in

¹ Paper No. 151, Journal Series, Georgia Experiment Station.

culture. It seems desirable, therefore, to work with freshly isolated cultures whenever it is possible. It must be noted, however, that of the 17 strains from Georgia, the majority of which were freshly isolated, three failed to form pycnidia in culture under any conditions. Nevertheless, it was possible by this method to demonstrate the ability of most of these isolates of *M. phaseoli* to form pycnidia, although only a single isolate of the fungus formed pycnidia on agar media.

TABLE 1.—*Dimensions of pycnosporos produced in culture on snap bean stems by various isolates^a of Macrophomina phaseoli*

Culture No.	Place of origin	Host	Dimensions of pycnosporos
			Microns
20	Georgia	Cowpea	
21	do	do	14.0–28.0 × 6.2– 8.4
24	do	Lima bean	14.0–28.0 × 5.9– 8.4
25	do	<i>Cassia nictitans</i>	19.6–28.0 × 8.1–11.8
43	do	do	
27	do	<i>Crotalaria intermedia</i>	19.6–26.6 × 8.4–10.6
28	do	<i>Lespedeza sp.</i>	16.8–22.4 × 6.2– 9.0
29	do	<i>Lactuca scariola</i>	16.8–33.6 × 7.8–11.2
45	do	Coffee weed	14.0–33.6 × 8.2–11.2
46	do	do	16.8–26.6 × 8.4–11.2
70	do	Sweet potato	
34	do	Snap bean	21.0–29.4 × 7.0– 8.4
35	do	do	16.8–33.6 × 6.2– 9.0
36	do	do	16.8–30.8 × 8.1– 9.8
37	do	do	14.0–22.4 × 7.0– 8.4
38	do	do	16.8–26.6 × 6.2– 9.8
75	do	do	11.7–22.4 × 8.4–11.2
31	California	Cantaloupe	16.8–33.6 × 7.8–11.2
32	do	Red Kidney bean	19.6–30.8 × 6.2–11.2
33	do	Bean	
42	do	Tomato	19.6–30.8 × 6.2–11.2
85	do	Sorghum	
30	Maryland	Sun flower	
41	Nebraska	Corn	19.6–30.8 × 7.8–11.2
88	do	Sorghum	
39	Texas	Cowpea	16.8–28.0 × 8.1–11.2
40	do	Sorghum	14.0–21.0 × 7.8– 9.8
84	do	do	
86	do	do	
87	Oklahoma	do	
89	do	do	
90	do	do	
91	do	do	
92	do	do	
93.	do	do	

^a Fifteen isolates failed to produce pycnidia in culture.

Inoculations with pycnosporos from culture No. 75 were made on Tender-green snap bean plants of all ages from the seedling stage to full maturity. Drops of a suspension of pycnosporos were placed on nonwounded stems and leaves. After allowing the inoculum to dry, the plants were placed in a moist chamber for 48 hours. They were then returned to benches in the greenhouse. Lesions appeared on the stems and leaves within two weeks,

resulting in the death of leaves and portions of branches and, ultimately, of entire plants. Excellent results were consistently obtained from inoculations with pycnospore suspensions when other methods of inoculation failed. Inoculations through the soil with oat cultures of *Macrophomina phaseoli* were successful only when the soil was inoculated prior to planting. The seedlings then became infected during emergence. It seems probable that more satisfactory results from inoculations with *M. phaseoli* on other crop plants might be obtained, if inoculations after emergence were made with pycnospore suspensions.

Since use of this pycnidial strain has greatly facilitated inoculation experiments with *Macrophomina phaseoli* on snap bean, it is offered to pathologists who are working on diseases of other plants caused by this organism in the belief that it may be helpful to them. Cultures may be obtained upon request to the Georgia Experiment Station.—E. S. LUTTRELL, Georgia Experiment Station, Experiment, Georgia.

Resistance to Cercospora apii Fres. in Celery (Apium graveolens var. dulce).—The early blight disease of celery caused by *Cercospora apii* Fres. is one of the most destructive and widespread diseases of this crop. It occurs throughout the United States, but is particularly destructive in the southern states. In Florida it is necessary to apply Bordeaux mixture or other protective fungicides 12 to 16 times during the growth of the crop to control the disease.

Variety trials over a period of years at the Everglades Experiment Station, Belle Glade, Florida, have failed to disclose a commercial variety of celery sufficiently resistant that it could be grown without the use of fungicides. However, in 1940-41 forty-eight foreign collections of celery were planted at Belle Glade, Florida, and these were examined for resistance to early blight. The collection of varieties had been made by plant explorers¹ of the United States Department of Agriculture and had been released to the Everglades Experiment Station by the Department of Agriculture. Among this collection of foreign celeries there were seven varieties originally from Turkey which remained free of early blight lesions and produced vigorous plants. This was in contrast to the extreme susceptibility of all commercial varieties from the United States.

The resistant Turkish celeries were dark green, hollow-petioled, strongly flavored plants, closely resembling celeriac. They probably were types used for cooking rather than as a green salad. There was no possibility of developing promising new varieties by selfing of single-plant selections from these varieties.

Since climatic factors operate against successful breeding of celery in Florida, a number of plants from the seven early-blight-resistant varieties were taken up and shipped to the Department of Plant Breeding at Cornell University in March and April, 1941. Only one of these plants flowered

¹ F. L. Wellman and H. L. Westover.

during the summer of 1941 and an attempt to hybridize it with Cornell 19 failed. The other plants produced no inflorescence during the summers of 1941 or 1942. After storage in a cold-frame during the winter of 1942-43, the plants were taken into the greenhouse late in January. Two plants flowered in May, 1943, and were caged with Cornell 19 plants which were in flower. Flies were used to effect cross-pollination. Seed was obtained during the summer from each of the caged plants, and it was later learned that the following four crosses were established: PEI 115557 \times Cornell 19, Cornell 19 \times PEI 115557, PEI 120875 \times Cornell 19, and Cornell 19 \times PEI 120875.

The F_1 plants were grown in a greenhouse at Cornell University during the winter of 1943-44 and flowered in April, 1944. Nine F_2 lines from these crosses were grown in the field at Belle Glade, Florida, during the winter of

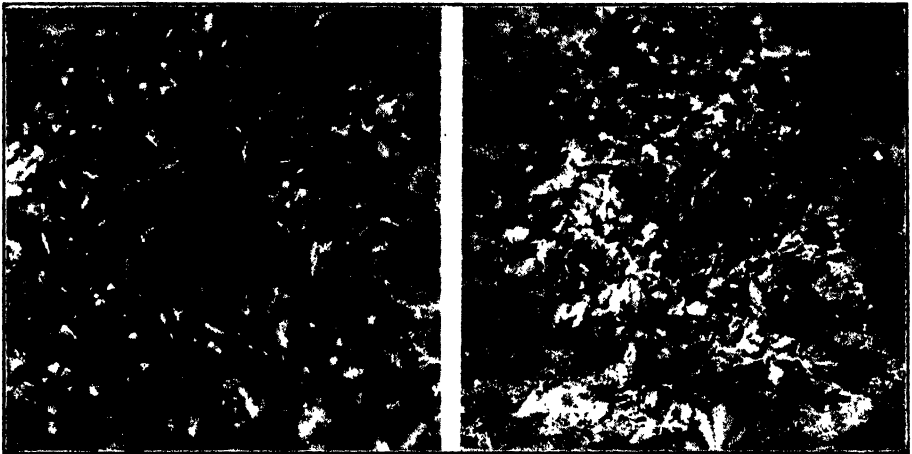


FIG. 1. An F_2 line of celery having a high degree of resistance to *Cercospora apii* (at left) and a susceptible commercial variety (at right).

1944-45. Most of the plants in these lines were very large, dark-green, hollow-petioled, and considerably more resistant to *Cercospora apii* than commercial varieties. There were a few plants which had segregated to solid petioles. These offered the opportunity long desired of combining disease resistance with a horticulturally acceptable plant type. Twenty-eight F_2 plants were selected as highly resistant to *Cercospora apii*. All of these had solid, fleshy petioles. Some were as green as Pascal celery, while others were a lighter green. Two selections were of the self-blanching type. The selected plants were shipped to Ithaca, N. Y., in March, 1945, and seed was obtained from seventeen of them during the summer. Nineteen other F_2 lines selected at Ithaca the previous summer also produced seed during 1945.

A planting of 35 F_3 lines was made at Belle Glade, Florida, during the winter of 1945-46. No protective fungicides were applied. Before the selections were made the plants were scored on the basis of apparent resistance to *Cercospora apii*. A score based on 10 points for complete susceptibility

was used. Lines scoring 1 or 2 had less blight than usually occurs in well-sprayed fields of commercial varieties. Scores above 3 would indicate too much blight, and if above 6 the celery would be worthless. On this basis three lines were scored 1; five were scored 2; ten were scored 3; twelve were scored 4; two were scored 5; two were scored 6; and one was scored 7. The Kilgore's Pride variety grown in the same plots was scored 8. Figure 1 shows a blight resistant F_3 line at the left, and a susceptible commercial variety at the right.

The resistance to *Cercospora apii* in these new celeries is due to more than a single genetic factor. There has been a segregation of plants intermediate between fully resistant and completely susceptible in the F_2 and F_3 generations. Even in the more resistant lines, there is evidence that the expression of resistance is modified by age and unfavorable cultural conditions. The green plants are usually more resistant than the self-blanching types. Most of the self-blanching segregates proved to be susceptible.

Green color in the petioles is dominant over the self-blanching habit. F_2 ratios approximating 3 green to 1 blanched indicate that the green color is due to a single dominant factor. The intensity of the green color varies and appears to be controlled by modifying factors.

Hollow-petiole, or pithiness, was fully dominant in the F_1 and showed an F_2 segregation approximating a 3 to 1 ratio at Ithaca, while at Belle Glade the percentage of solid-petioled plants in the F_2 seemed to be much lower. This suggests that there may be a modifying factor or factors affecting the expression of the 3 to 1 ratio for pithiness under certain climatic or cultural conditions.

Red color which occurs in the petioles of some lines is inherited as a simple dominant. The F_2 generation showed a segregation of 3 red to 1 non-red. This color is expressed most strongly near the base of the petioles.

The inheritance of the factors for ribbed or smooth petioles has not been studied, although it was observed that the majority of the lines are more or less ribbed. A few of the F_3 selections had extremely smooth petioles.

The quality of several of the F_3 selections is high. They have very heavy, crisp petioles, and are not stringy. In some lines the base of the petiole is somewhat flared, and thickened. Rib length varies from 7 to 15 inches in the different lines which have been selected. The fact that the best selections are of the green sorts should be no hindrance to the development of new commercial varieties from them. Pascal-type celeries have been gaining in popularity and may ultimately supplant the self-blanching celeries in consumer preference. The new celeries have an unusual flavor which is delicious in mixed green salads.—G. R. TOWNSEND, Everglades Experiment Station, Belle Glade, Florida, and R. A. EMERSON, Department of Plant Breeding, and A. G. NEWHALL, Department of Plant Pathology, Cornell University, Ithaca, N. Y.

SOIL ACTINOMYCETES APPLIED TO BANANA PLANTS IN THE FIELD¹

CLIFFORD H. MEREDITH

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In November, 1942, the writer (1) observed that an actinomycete, a *Streptomyces*, isolated from a compost heap in a banana area near Port Maria, Jamaica, British West Indies, was antagonistic to *Fusarium oxysporum* Schl. var. *cubense* (E.F.Sm.) Wr. and Rg., the Panama disease fungus. A survey was then made to determine the prevalence of organisms antagonistic to *F. oxysporum cubense* in banana soils in Jamaica (2). One hundred and twenty-two organisms having such antagonism in culture were isolated: 66 were slightly antagonistic, 39 antagonistic, and 17 very antagonistic. Five of the actinomycetes classed as very antagonistic, numbers 412, 512, 569, 976, and 1182 in table 1, were used in field experiments and are

TABLE 1.—*The antagonistic effect of various actinomycetes on the growth of Fusarium oxysporum cubense on Orange River soil-solution agar*

Actinomycetes ^a	Distance planted from Fusarium	Fusarium growth towards actinomycetes		
		6 days	20 days	35 days
	cm.	cm.	cm.	cm.
1182	1.3	0.5	0.8	0.6 ^b
569	1.3	0.7	0.7	0.7
341	1.3	0.9	1.0	1.0
448	1.3	1.0	1.1	1.1
344	1.2	1.1	1.0	1.1
487	1.1	1.1	1.1	1.1
462	1.3	1.0	1.1	1.3
976	1.3	1.0	0.9	1.3
153	1.3	1.1	1.3	1.3
412	1.3	1.0	1.5	1.3
512	1.5	1.2	1.0	1.5
947	2.0	2.0	2.1	1.9
671	1.4	1.4	3.4 ^c	3.6
45	1.1	3.6	5.3 ^d	..
918	1.4	1.4	5.6	..
188	1.4	3.0	6.0	..
1	1.1	2.8	6.3	..
None	Check	3.1	5.5	..
None	Check	2.9	5.4	..
None	Check	2.9	5.8	..

^a Isolated from fields outside the Orange River area.

^b Shrinkage indicates lysis.

^c Where the growth is greater than the distance apart the Fusarium crossed over the line of the actinomycetes.

^d The Fusarium had grown to the bottom of the test tube.

referred to in this paper as general actinomycetes. The antagonism of some of these isolates was substantiated by Thaysen and Butlin (4) who obtained cultures of these actinomycetes and of *F. oxysporum cubense* from the National Type Culture Collection in England.

¹ Acknowledgment is due the Jamaica Banana Producers' Association and the planters in the Parish of St. James for support in this research.

In order to test these general actinomycetes under field conditions plans were made with the Director of Agriculture for Jamaica for an extensive field experiment at the Orange River Experiment Station of the Department of Agriculture in one of the chief banana growing areas in the island. The site selected for the experiment was known to be heavily infested with *Fusarium oxysporum cubense*, as banana plants with Panama disease had been removed only a few months previously (3).

MATERIALS AND METHODS

The general actinomycetes were grown in test tubes with *Fusarium oxysporum cubense* on Orange River soil-solution agar at 1.0 to 2.0 cm. apart. None of them exhibited as much antagonism on this medium as on agar made from their own soils, although one gave almost equal results. These actinomycetes were increased for use in the field.

Samples of Orange River soil were examined for antagonistic actinomycetes. Of those found, 1 was antagonistic and 7 were slightly antagonistic. The 1 antagonistic and 3 of the slightly antagonistic culture strains were increased, for field tests, by growing them on guinea grass and soil in liter flasks. The contents of the flasks were mixed together, thinned with water, and sprinkled over the soil and suckers before the suckers were planted. Suckers were obtained from the Orange River property.

There were five different plot treatments with 15 stools in each bed and 7 replications. The treatments were as follows:

Treatment No. 1. Check. This was the customary clean culture method of banana growing in the area.

Treatment No. 2. Mulch. Green guinea grass was cut from contour strips and placed around the stools.

Treatment No. 3. Suckers and soil were inoculated at the time of planting with a mixture of the 5 general actinomycetes.

Treatment No. 4. This was the same as treatment No. 3 with mulch added.

Treatment No. 5. Suckers and soil were inoculated at the time of planting with a mixture of the four actinomycetes from Orange River soil and mulch was added.

Green guinea grass was applied three times during the year on the mulched beds.

Suckers were planted on May 23, 1944. Growth measurements consisting of height of plant, circumference of base, width of widest leaf, and number of leaves were made on August 8 and October 10, 1944, and on May 9, 1945. Record of Panama disease occurrence was also kept.

RESULTS

General Actinomycetes

Mean growth measurements of plants in plots treated with general actinomycetes were higher than mean growth measurements of plants in check

plots in August and October readings. The difference in height in October was statistically significant.

Plants in plots treated with general actinomycetes gave higher mean growth measurements in August and October than plants in plots treated with general actinomycetes and mulch. Three of these differences were significant in August and two in October (Table 2).

TABLE 2.—Means of growth measurements for banana plants

Treatment	Height	Circumference	Width of leaf	Number of leaves
	<i>inches</i>	<i>inches</i>	<i>inches</i>	
<i>August 8, 1944</i>				
Cheek	34.07	4.96	9.39	8.64
Mulch	29.08	4.69	8.71	7.87
Actinomycetes	34.51	5.23	10.24	9.11
Actinomycetes plus mulch	30.69	5.05	9.22	8.10
Orange River actinomycetes plus mulch	35.51	5.32	10.63	8.83
F	3.16 ^a	1.2	3.42 ^a	4.22 ^b
M.S.D.	4.35		1.23	0.73
<i>October 10, 1944</i>				
Cheek	57.33	8.13	14.67	10.06
Mulch	57.02	7.87	14.57	10.47
Actinomycetes	65.14	9.04	16.05	10.09
Actinomycetes plus mulch	59.70	8.29	15.56	10.62
Orange River actinomycetes plus mulch	70.84	9.60	17.18	10.73
F	8.45 ^b	3.07 ^a	3.2 ^a	1.0
M.S.D.	6.03	1.1	1.73	

^a Significant value, 2.78.

^b Highly significant value, 4.22.

The Effect of Mulch

Plants in plots treated with mulch alone were usually smaller than unmulched plants in check plots.

Plants in plots treated with general actinomycetes and mulch were usually smaller than plants in plots treated with general actinomycetes without mulch. The difference in the number of leaves in August was significant (Table 2).

Orange River Actinomycetes

Banana plants in plots treated with Orange River actinomycetes on mulch gave the highest readings in three out of four measurements in August and in all measurements in October.

In August, plants treated with Orange River actinomycetes on mulch gave significantly higher measurements in height, leaf width, and number of leaves than those treated with mulch alone and those treated with general actinomycetes on mulch. Plants treated with Orange River actinomycetes also gave significantly higher measurements in leaf width than those in check plots.

In October, plants treated with Orange River actinomycetes on mulch gave significantly higher measurements in height, circumference, and width of leaf than those in check plots and in plots treated with mulch alone. They also gave significantly higher measurements in height and circumference than plants in plots treated with general actinomycetes on mulch (Table 2).

Panama Disease Occurrence

During the first five months symptoms of Panama disease appeared in 26 stools (Table 3). These stools were distributed as follows: Check, 2;

TABLE 3.—*Occurrence of Panama disease in the check, mulched, and actinomycete-treated beds from date of planting, May 23, 1944, to May 9, 1945*

Treatment	Missing stools	Plants with Panama disease		Healthy stools, May, 1945
		May to Oct., 1944	Oct. to May, 1945	
	No.	No.	No.	No.
Check	3	2	71	29
Mulch	3	5	75	22
Actinomycetes	2	12	53	38
Actinomycetes plus mulch	3	3	73	26
Orange River actinomycetes plus mulch	1	4	76	24

Mulch, 5; General actinomycetes, 12; General actinomycetes with mulch, 3; and Orange River actinomycetes with mulch, 4. Such early attacks are generally attributed to infection in the suckers before they are planted. During the last seven months 348 stools went down with Panama disease. The final distribution of diseased stools was: General actinomycetes, 53; Check, 71; General actinomycetes with mulch, 73; Mulch, 75; and Orange River actinomycetes on mulch, 76.

DISCUSSION

The general actinomycetes which had been classed as "very antagonistic" on their own soil had less effect on growth of banana plants in Orange River soil than Orange River actinomycetes, in spite of the fact that the latter had been classed as "antagonistic" and "slightly antagonistic." This fact raises the question of the essential nature of such antagonism.

Mulch, in these experiments, appeared to be detrimental to the growth of banana plants. This is in agreement with the fact that clean culture methods of banana cultivation have been found to be most satisfactory in Jamaica.

The plants in plots treated with Orange River actinomycetes and mulch gave the highest measurements the greatest number of times. The detrimental effect of mulch was apparently overcome to some extent by the presence of antagonistic actinomycetes functioning on their own soil.

The Orange River plot on which these experiments were carried out was known to be heavily infested with *Fusarium oxysporum cubense*. Some of the plots from which the "very antagonistic" general actinomycetes were taken were known to be areas in which the spread of the disease had been less rapid than was the general rule. Even though the disease was present, over a period of several years fewer plants had succumbed to it than in nearby areas. The nature of this "natural resistance" was not known. Waksman (5) states that "20 to 40 per cent of all actinomycetes are known to be capable of producing antibiotic substances." This might have some bearing on the case as the most antagonistic actinomycetes came from fields where natural resistance was high.

Although the plots treated with the general actinomycetes produced the largest number of plants free from Panama disease, the difference was not significant because of the wide variation in the number of diseased plants in the replications within the treatments.

There was also indication that the mulch increased the occurrence of Panama disease but again the difference was not significant. It must be pointed out that only one application of actinomycetes was made in these experiments at the beginning when the plants were set out.

SUMMARY

Significant differences in growth measurements were found to exist between plants grown on plots treated with actinomycetes of varying degrees of antagonism and those grown without such treatment.

Treatment with "antagonistic" and "slightly antagonistic" actinomycetes on their own soil resulted in greater growth increases in banana plants than did the treatment with "very antagonistic" actinomycetes from other soils.

Growth measurements of plants treated with mulch did not compare favorably with those of unmulched plants.

Plots treated with "very antagonistic" actinomycetes from other soils produced fewer plants with Panama disease than did plots treated with "antagonistic" and "slightly antagonistic" actinomycetes from Orange River soil.

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HELMINTHOSPORIUM ROT OF TOMATO FRUITS

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The demand for fresh tomatoes in the United States from November until May is greater than the domestic supply. The importation of tomatoes during this period is principally from Mexico, Cuba, and Haiti. Mexico alone shipped more than 8,000 carloads of "green-wrapped" tomatoes into the United States and Canada during the 1944-45 season (9, 10, 11). The producing areas are largely confined to three States in Mexico; Sonora and Sinaloa on the west coast with an estimated 49,000 acres planted for the 1945-46 season, and Tamaulipas on the east coast with 6,000 acres.

In the past 11 years United States plant quarantine inspectors have occasionally intercepted fruits with decayed spots that were unlike the usual tomato diseases encountered in the domestic crop. The first interception of this disease was made from a shipment of tomatoes from Mexico in April, 1934, by J. R. Wood of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. Since that time 16 additional interceptions of diseased fruit from Mexico, 2 from Haiti, and 1 from British Guiana have been made by plant quarantine inspectors.

The diseased specimens were sent to John A. Stevenson of the Division of Mycology and Disease Survey, U. S. Department of Agriculture, who suggested that the disease and causal organism be studied.

DESCRIPTION OF DISEASE

Specimens of the rot intercepted at ports of entry were characterized by circular to irregular areas on the fruit, 10-30 mm. in diameter, covered by a dense web of dark gray to almost black mycelium which completely obscured other spot characteristics.

The fungus was isolated from the intercepted specimens and the isolates were used to inoculate mature green tomatoes, and from these the characteristics of the rot were observed.

The lesions were flattened to slightly sunken, at first circular, but becoming irregular as the decay advanced. Considerable variation was found in the surface coloring of the lesions, but in general it was distinct from other tomato rots. The tissue about the point of infection was usually dark or black. The most variable character was the extent of the cream-colored area surrounding the dark center. It was nearly always present at temperatures of from 60° to 80° F. and served as a striking diagnostic character (Fig. 1, A, B). A darker area ranging from bay to burnt sienna to mahogany red³

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³ The descriptive color terms used in this paper are those given in Robert Ridgway's Color Standards and Color Nomenclature, 1912.

surrounded the cream-colored area. Occasionally there were lesions that advanced slowly at 70° F., in which the cream-colored area never developed (Fig. 1, C). Such lesions resembled those caused by *Alternaria*. At lower temperatures, especially at 50° F., the spot was poorly defined; the fungus penetrated the tomato so slowly that at the end of a week the tissue was not completely killed, but had a bronzy appearance (Fig. 1, D). When these

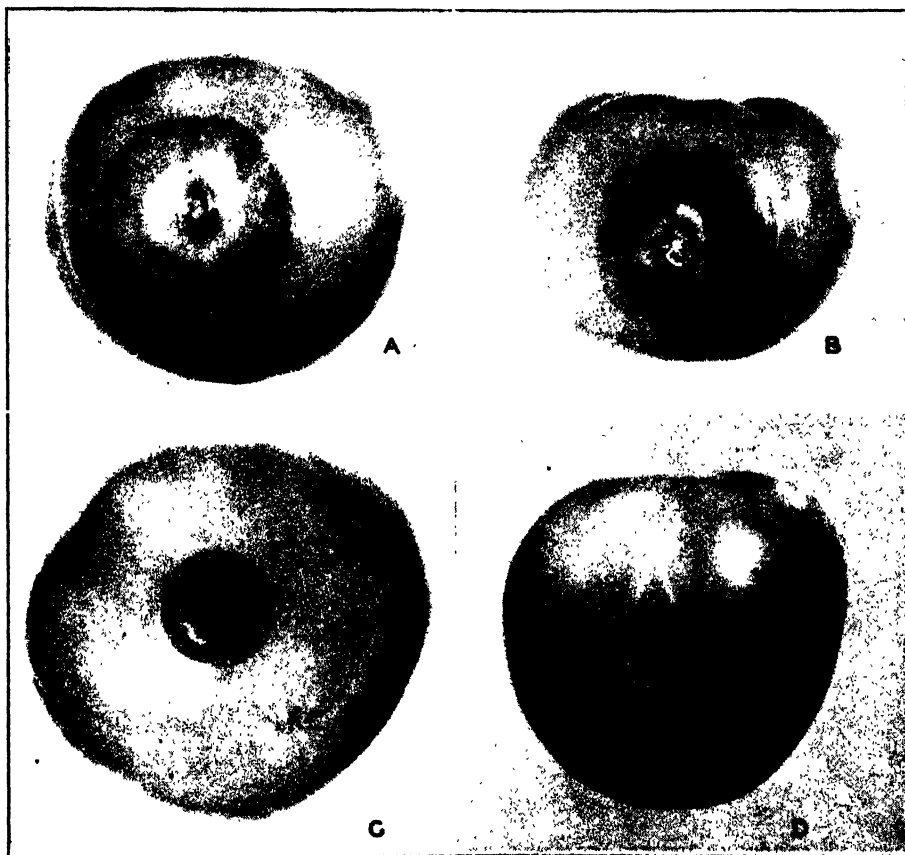


FIG. 1. Tomato inoculated when mature green with *Helminthosporium carposaprum*. A and B, typical decay at 70° and at 80° F.; C, variation also produced at 70° F., which resembled *Alternaria* rot. D, symptoms of infection produced at 50° F. Photographs by Lilian A. Guernsey.

fruits were transferred to higher temperatures the lesions gradually became like those developed at higher temperatures.

The rot consisted of a firm spongy mass of decayed tissue which extended deeply into the fruit but caused no offensive odor. When fruits with advanced decay were cut, dark gray mycelium was evident through the necrotic tissue.

MORPHOLOGY OF THE FUNGUS

Sporiferous areas can usually be located on diseased tomatoes by their

darker color, which is due to the dark-colored conidiophores borne singly or in clusters (Fig. 2, B) on the skin of the tomato. Conidiophores are erect, many-septate, $140-500 \times 6-10 \mu$, sometimes tapering to a width of $4-6 \mu$ at the apex which is often paler. The base of the conidiophore (Fig. 2, A) con-

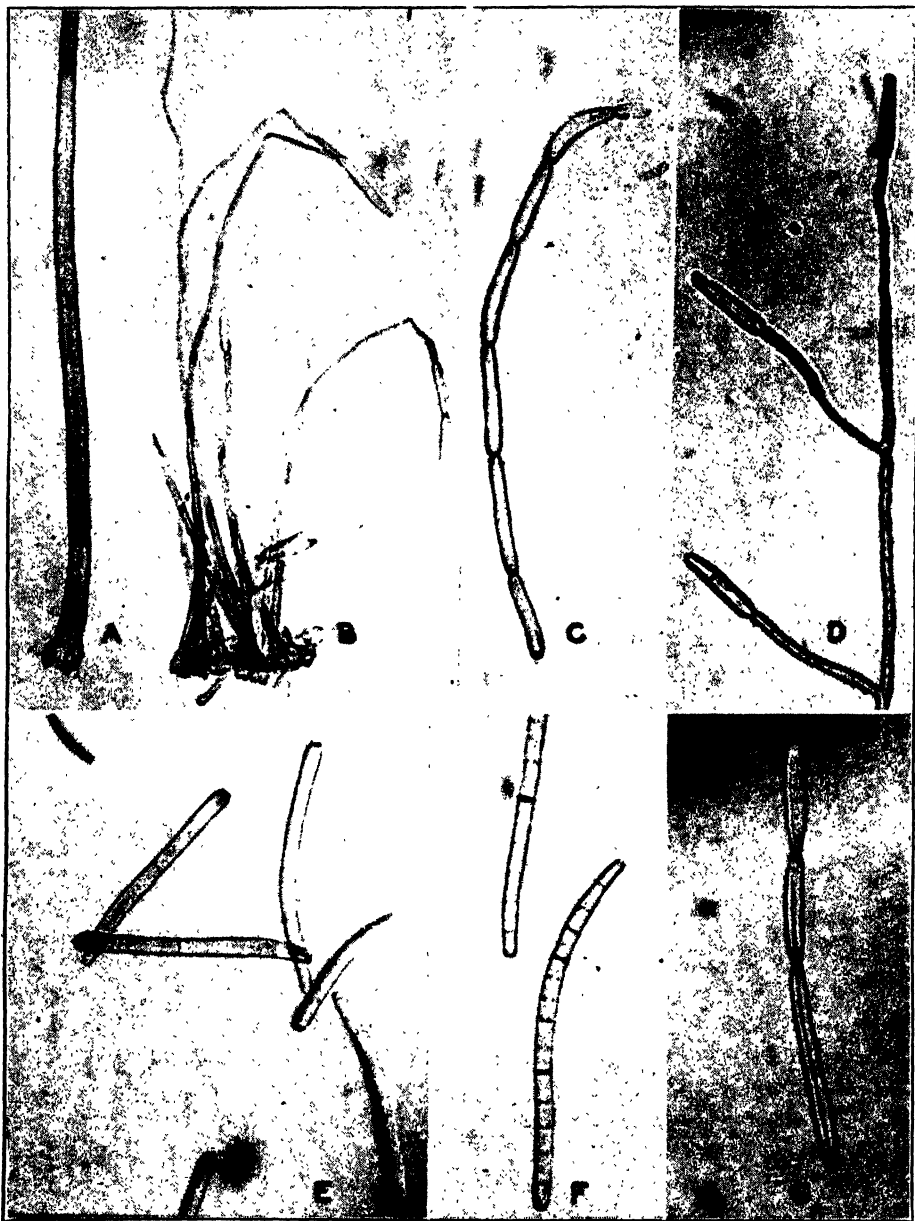


FIG. 2. *Helminthosporium carposaprum*: A, enlarged conidiophore with bulbous base; B, typical conidiophores with conidia, developed on the skin of tomato; C, conidial chain; D, type of conidiophore developed in culture; E, F, and G, variations in conidia. Photographs A and B by Marcel Foubert, C through G by Lilian A. Guernsey.

sists of a thick-walled, dark brown, bulb-like cell, $12-16 \times 10-12 \mu$. Under conditions of high humidity conidiophores are produced as lateral or terminal branches of the hyphae (Fig. 2, D). These are usually narrower ($4-6 \mu$) and may be very short projections from hyphae, or as long as the conidiophores arising from a fruit. Geniculate conidiophores were not observed.

Conidia are usually cylindrical, occasionally tapering, with an obtuse to rounded basal end and apex. The apex tends to become obtuse when secondary conidia are formed there. Long chains of acropetally produced conidia are frequently observed (Fig. 2, C). The conidia are sub-hyaline to dilute olivaceous, straight or curved, 1-15 septate, $28-220 \times 6-12 \mu$, usually $120-160 \times 8-10 \mu$ (Fig. 2, E, F). Budded conidia are usually smaller, paler in color and often continuous (Fig. 2, G). Spores germinate by the production of one or two polar germ tubes which pass through the inconspicuous hila that may be formed at both basal and apical ends of spores.

SYSTEMATIC TREATMENT

From specimens received in 1934 and 1938, John A. Stevenson recognized the causal fungus to be distinct from *Helminthosporium tomato* Ell. and Barth. (3) which is reported to occur on decayed tomatoes. In 1942 Miss Edith Cash working on two other specimens reported that conidial dimensions were similar to those of *Helminthosporium lycopersici* Maub. and Roger, but that conidiophore length was different. *H. lycopersici* was described from tomato leaves in Africa by Maublanc and Roger (5) July 10, 1936. Roldan (6) used the same binomial for a fungus which he described from leaves of *Lycopersicon* from Luzon, Philippine Islands, in the same year. The account of his fungus appears in the June, 1936, issue of the Philippine Journal of Science, which was not actually published until September 2, 1936, as noted in the table of contents of volume 60, for 1936. Maublanc and Roger, therefore have priority in the use of the name. It has not been possible to determine whether the two fungi are synonyms due to lack of specimens for comparison and to the inadequacy of the description of Maublanc and Roger.

Comparison of Roldan's description and illustrations with the *Helminthosporium* under investigation here revealed differences in size, shape, and color of the fructifications. A specimen of *Helminthosporium rhopaloides* Fres. reported on dead tomato stems has been examined and found to have shorter and darker conidia and conidiophores (7; 8, Fig. 831).

For the fungus under discussion, which does not appear to have been described, the name *Helminthosporium carposaprum* is proposed, the specific epithet being suggestive of the fruit rotting effect.

DIAGNOSIS⁴

Helminthosporium carposaprum Pollack sp. nov.

Maculae in fructibus *Lycopersici* esculenti plerumque per mycelium prae-

⁴ The authors wish to express their appreciation to Miss Edith K. Cash for her help in the preparation of the Latin diagnosis, and to John A. Stevenson for his suggestions.

crassum fumentem usque nigellum vestitae; conidiophora in peripheria maculae, singula vel caespitosa, fusca, recta, multi-septata, $140-500 \times 6-10 \mu$ ad apicem $4-6 \mu$ crassum, pallidum attenuata, basi obscuriori, pachypileo, bulboso; conidia acrogena, cylindracea, ad basim et apicem obtusa usque rotundata, interdum attenuata, recta vel curvata, subhyalina usque dilute olivacea (obsuro olivaceo-bubalina Ridgway), $1-15$ septata, $28-220 \times 6-12 \mu$ frequenter $120-160 \times 8-10 \mu$ plerumque catenulata.

Lesions on tomato fruit predominantly cream-buff with a darkened area around the infection point, with the outer zone of the spot bay, burnt sienna, or mahogany red. Spot characteristics usually masked by an overgrowth of a thick weft of dark gray to almost black mycelium.

Conidiophores appearing around the edge of the spot, singly or in clusters, dark, erect, many-septate, $140-500 \times 6-10 \mu$, sometimes tapering to a width of $4-6 \mu$ at the relatively paler apex, with a dark, thick-walled, bulbous basal cell, $12-16 \times 10-12 \mu$, attached to the skin of the fruit.

Conidia produced at the tips of the conidiophores only, usually in long chains, cylindrical with obtuse to rounded base and apex, sometimes tapering, straight or curved, sub-hyaline to dilute olivaceous (dark olivaceous buff according to Ridgway), $1-15$ septate, $28-220 \times 6-12 \mu$, most often $120-160 \times 8-10 \mu$, germinating by means of one or two polar germ tubes passing through the hila.

On fruit of *Lycopersicon esculentum* grown in Mexico, Haiti, and British Guiana. Specimens are deposited in the Mycological Collections, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture (Type No. 71481 on inoculated tomatoes).

CULTURAL RESPONSES

Helminthosporium carposaprum grew readily on various nutrient media such as cornmeal, string bean, prune, and potato-dextrose agar. The fungus also flourished on autoclaved green stems of tomato and of a coarse wild grass. Growth at 70° to 80° F. was rapid and the fungus sporulated within 5 to 7 days. Irregularities in the size and shape of spores and conidiophores are common in culture. Conidiophores arise only as lateral or terminal outgrowths of hyphae and are scarcely distinguishable from the mycelium. The conidia are smaller in culture than on the tomato fruit, and frequently produce secondary spores which in turn often produce still other spores. Long chains of these spores are common in culture as well as in nature. Similar tendencies toward catenulation have been reported by Drechsler (2), by Berg (1), and by Olive (4). Another characteristic in culture is the production of sporophoric processes from the apices of spores, and chains of spores interspersed with these sporophoric structures are often seen. This characteristic has also been reported by Drechsler (2) and by Berg (1).

TEMPERATURE STUDIES

In the temperature studies of the fungus in the fruit, the temperature range was primarily confined to those encountered in commercial handling.

However, inoculated tomatoes were stored at 32°, 36°, and 40° F. to observe the possibility of infection at these lower temperatures. This wider range was also employed in the studies on nutrient media.

ON TOMATO FRUIT

Growth measurements of the fungus lesions were made in order to obtain the rate at which decay developed at 80°, 70°, 60°, 55°, and 50° F. Mature green tomatoes used in this study were either purchased in a Washington, D. C., market or grown at the Plant Industry Station. A series of four tests were made using from 12 to 24 fruits for each temperature in each test. The fruits were washed in 50 per cent alcohol, dried, and inoculated by inserting bits of mycelium in small oblique wounds. One inoculation was made per fruit. The fruits were wrapped and stored at the above temperatures. Measurements were made after 7, 14, and 21 days had elapsed. Figure 3 shows the effect of temperature on the rate of development of the lesions.

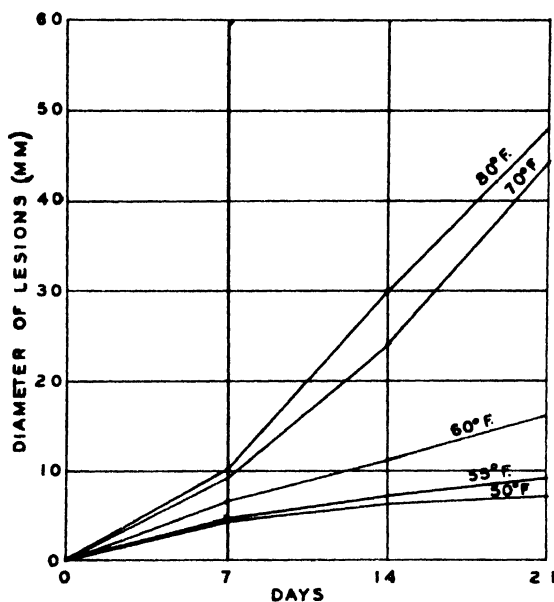


FIG. 3. Effect of temperature on rate of development of lesions on mature green tomatoes produced by *Helminthosporium carposaprum*.

Decay lesions developed slowly at all temperatures during the first week. At the end of 14 days and thereafter, there was a sharp contrast between development at the higher temperatures of 70° and 80° and that at 60° F. and below, as is shown in figure 3. The indications are that a sizable rot can develop on mature green tomatoes during shipment and ripening when temperatures are about 70° F. or above. However, the fungus was tolerant of lower temperatures. While decay at 60° F. and below was slow, it was in such a stage of development that the lesions enlarged rapidly when the fruits were removed to the ripening room at 70° F. The fruits were weakened by

subjection to 32°, 36°, and 40° F.; no infection was evident at 32° or 36° during 30 days but there was slight infection at 40° F. after 10 to 14 days.

ON AGAR PLATES

Measurements to obtain the rate of growth of the fungus in culture were made at 80°, 70°, 60°, 55°, 50°, 40°, 36°, and 32° F. Five to 10 Petri plates with 20 cc. of Czapek's agar were used at each temperature and the test was repeated. The inoculum, consisting of a small amount of mycelium, was planted in the center of the agar and the plates were stored immediately. Measurements of the diameter of the colonies were made after 7, 14, and 21 days.

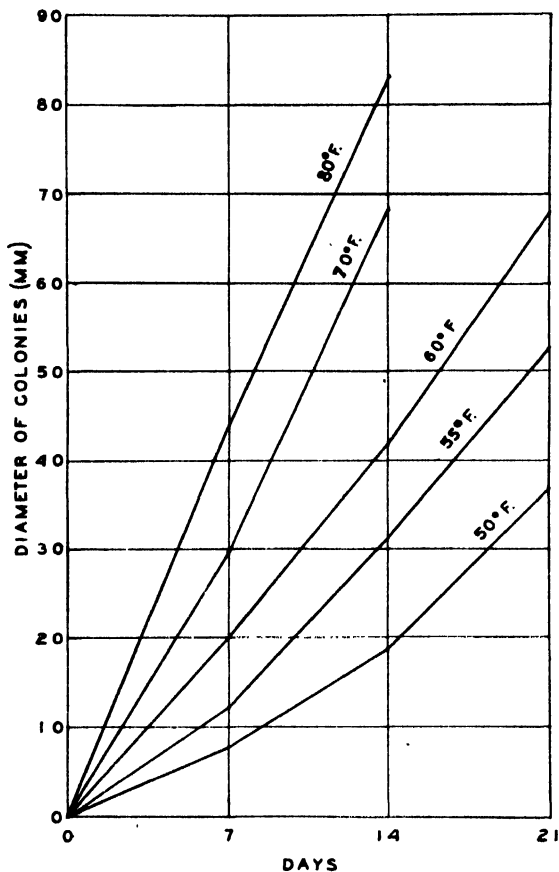


FIG. 4. Effect of temperature on growth rate of *Helminthosporium carposaprum* on Thaxter's agar in Petri plates.

Figure 4 shows that the *Helminthosporium* growth rate was approximately twice as rapid at 80° as at 60° F., fully twice as rapid at 70° as at 55° F. and about twice as rapid at 60° as at 50° F. No measurable growth occurred at 40°, 36°, or 32° F. during 21 days. However, when examined with a hand lens, the inoculum in those plates stored at 40° was found to be

covered with new growth, while at 36° and 32° F. it seemed dormant. Colonies developed when these plates were transferred to room temperature.

A comparison of the diameter of the fungus colonies on agar with the diameter of the lesions on the fruit at the end of 3 weeks showed that the colonies on agar increased from 2 to 5 times as much as the lesions on the fruit at corresponding temperatures.

INFLUENCE OF TEMPERATURE ON SPORE GERMINATION

Spores were placed in sterile water and in diluted prune juice and stored at 80°, 70°, 60°, 55°, 50°, 40°, 36°, and 32° F. Within 24 hours germination had started at 50° F. and above. By the end of 72 hours, some spores were germinating at 40° F. and after 9 days about 35 per cent of these spores had germinated. Spores held at 36° and 32° were found to be dormant even after 22 days.

EFFECT OF FREEZING

Vigorous cultures of the *Helminthosporium* on Thaxter's agar slants were placed at 16° F. to determine the effect of such a low temperature on the survival of the fungus. Transfers from withdrawals after 6, 7, 9, 14, 21, and 51 days gave viable cultures, but cultures exposed for 63 days were dead.

INOCULATION EXPERIMENTS

Tomato Plants

As most species of *Helminthosporium* are associated with leaf-spotting diseases it seemed of interest to test the pathogenicity of the tomato fruit-rotting fungus on tomato plants. Potted plants that had started to bloom were used for these tests. The leaves were punctured with a needle, making one or two groups of holes in each leaflet. The inoculating was done by atomizing an aqueous spore suspension on the plants. Inoculated and non-inoculated plants were then placed in a humid chamber designed to keep the air automatically saturated with water.

Extensive spore germination was observed in 24 hours in the unused portion of the inoculum which was left in the humid chamber with the plants. The margin of the punctures in the leaves became blackened after 24 hours, but there was never any manifestation of disease.

The experiment was repeated, and for part of the material to be inoculated plants were selected that were not bound and physiologically weakened, as shown by a general yellowing of the foliage. In the second test the vigorous plants (Fig. 5, B) again failed to develop the disease but necrotic lesions developed about the punctures of the devitalized plants (Fig. 5, A), from which the *Helminthosporium* was recovered. These experiments indicate that the fungus did not cause a leaf spot of tomato when the plants were in a reasonably good state of vigor. In addition to the leaf inoculations, mats of mycelium were inserted into the plant stems, but failed to become established or cause lesions.

Tomato Fruits

Mature green tomatoes, when inoculated by inserting a mat of mycelium, developed a sizable rot by the time the fruit was ripe, as is shown in the discussion on temperature studies.

To test a smaller amount of inoculum, mature green tomatoes were punctured with a sterile needle and dipped in an aqueous spore suspension. Test lots were stored at 40°, 50°, 55°, 60°, and 70° F. Infection was erratic and slow. Lesions 1 to 2 mm. in diameter were evident on some fruits stored at 60° F. for 9 days.



FIG. 5. A, Leaf from devitalized tomato plant showing *Helminthosporium* lesions forming around points of inoculation. B, Leaf from vigorous plant failed to develop the disease. Photograph by Lilian A. Guernsey.

Other Plant Products

To study the behavior of this fungus further, fruits from certain other plants were inoculated by inserting a mat of mycelium under the skin, and storing them in moist chambers at 60° F.

Slow decay developed on York Imperial apples (*Malus sylvestris* Mill.), scallop squashes (*Curcubita pepo* Mill.); moderate decay developed on egg-plant fruit (*Solanum melongena* L.) and bell peppers (*Capsicum frutescens* var. *grossum* Bailey). The fungus was unable to cause infection in orange (*Citrus sinensis* L. Osbeck) or lemon (*Citrus limon* (L.) Burm. f.) fruits.

The decay lesions on the apple fruits were firm and remained small, about 10 mm. in diameter, even though the fruits were held until they broke down

from over-ripeness. Round, firm lesions from 6 to 8 mm. in diameter developed on scallop squashes in 9 days. The firm, round sunken lesions developed slowly on eggplant at first, measuring 10 to 15 mm. in diameter in 9 days, but the decay continued to spread and eventually consumed a large part of the fruit. Decay developed more rapidly on bell peppers with lesions of irregular patterns.

DISCUSSION

The studies on *Helminthosporium carposaprum* indicate that it is a weak parasite both in the field and on mature green tomato fruits during transportation and subsequent ripening. The fungus failed to produce leaf spots or stem lesions on vigorous tomato plants in spite of wounding and favorable infection conditions. Lesions developed, however, around puncture wounds when plants in a low state of vigor were inoculated. The fruits appear to be more susceptible than other parts of the plant, but a sizable amount of inoculum is necessary to produce a decay that will affect the marketability. It seems probable that in the field the fungus grows on weakened plant tissue and debris and that this material then becomes a source of infection for tomato fruits.

From the limited reports at hand, *Helminthosporium carposaprum* does not appear to cause a serious disease. From observations of infection at 40° and 50° F. it seems probable that infection is encouraged by low temperatures and light frosts unfavorable to the fruit in the field, such as are encountered in Mexico during the growing and harvesting season (9, 10, 11). It is believed that the interception of tomatoes with sizable decay at ports of entry is the result of careless sorting and grading rather than the rapid development of the fungus in transit.

From inspectors' records for the past eleven years, there seems to have been no extensive increase in this disease, and no record of its presence in the United States. In view of this, and from the results of these studies, it seems unlikely that this fruit rot will reach serious proportions in the United States.

SUMMARY

The study of an undescribed rot of tomato fruits intercepted in imports from certain tropical or subtropical regions is reported. The cause of the disease, demonstrated by pure culture inoculations, is an undescribed species of *Helminthosporium*. This is now described under the name *Helminthosporium carposaprum*.

Inoculation of tomato stems, foliage, and mature green fruit indicated that the fungus is a weak parasite.

The fungus caused slow to moderate decay when inoculated into the harvested fruits of eggplant, bell pepper, scallop squash, and apple, but not of orange or lemon.

Temperature studies of the fungus on tomato fruits and on artificial media indicated tolerance to a wide range of temperature. Of the tempera-

tures tried, the best growth was obtained at the higher temperatures, 70° and 80° F. There was slight development of the fungus at as low as 40° F. and the fungus remained viable after 51 days at 16° F.

The *Helminthosporium* fruit rot of tomato does not appear to have increased significantly during the past 11 years. Although tomatoes from those areas in which the disease is found have been imported during this time there is no record of occurrence of the disease in the United States.

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THE EFFECT OF FUNGICIDAL SEED TREATMENTS ON GUAYULE SEEDLING EMERGENCE

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INTRODUCTION

In 1942 and 1943 the Guayule Emergency Rubber Project, under the direction of the U. S. Forest Service, established several large guayule (*Parthenium argentatum* Gray) nurseries in California for the production of planting stock. Pre-emergence losses in these nurseries and especially those located at Salinas where most of the field planting stock was produced, ranged from moderate to heavy. The average seedling emergence varied from 40 to 50 per cent of the germinable seed sown and seldom exceeded 75 per cent. Because of poor and unsatisfactory emergence in 1943 approximately one-fourth of the nursery beds had to be resown in two of the Salinas nurseries. Damping-off fungi, *Pythium* and *Rhizoctonia*, have been reported (8) as the principal cause of pre-emergence losses. Post-emergence damping-off did occur but the losses were comparatively low in 1942 and 1943 when the guayule nurseries were being operated on a large-production scale.

As a part of the general research program to facilitate the guayule rubber emergency production program, investigational work was undertaken to determine the cause or causes of poor seedling emergence and to develop control measures. The results of greenhouse, nursery, and field tests are herein reported.

DAMPING-OFF FUNGI

Various fungi have been isolated by the writer and others (4, 5, 7) from young guayule seedlings affected with damping-off and seedling root rot. Of these, *Pythium ultimum* Trow was predominant. However, various *Fusaria* were isolated frequently and a *Rhizoctonia* sp. was isolated occasionally. Several other fungi, considered to be non-pathogenic and commonly obtained in culture from plants affected with damping-off, were also obtained.

Pathogenicity tests were made in the greenhouse with many of the isolates obtained from diseased seedlings. All of the isolates of *Pythium ultimum* and *Rhizoctonia* tested were found to be virulent pre-emergence and seedling root-rot pathogens. The results were inconclusive as to the pathogenicity of the several *Fusaria* isolated. *Phytophthora drechsleri* Tucker, which was isolated from root-rot lesions on older plants (7), was found to attack and kill emerging guayule seedlings but was not isolated from damped-off nursery seedlings.

Of the many fungi associated with damping-off in the nursery and field

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sowings in California, *Pythium ultimum* is considered the most important. However, the sum total of the losses caused by the other fungi may be much greater than now suspected.

SEED

Both threshed and unthreshed guayule seed have been used in nursery and direct field sowings. However, most of the seed sown for production purposes has been unthreshed. Threshed seed has been used in both nursery and direct field sowings in large scale tests and was found to be equal or superior to the unthreshed seed. Both sorts of seed were used in testing various fungicidal seed treatments. The unthreshed seed (2, 6) consists of an achene with attached floral parts, and in threshed seed the accessory floral parts have been mechanically removed from the achene.

The percentage of filled or potentially viable seed varied greatly between different lots and was usually lower in the unthreshed than in the threshed seed. In the threshing and cleaning process many of the unfilled seed were removed. The percentage of filled seed usually ranged from 30 to 40 in the unthreshed lots, and from 60 to 70 in the threshed lots. These wide variations in filled seed, as well as in the percentage of germinable seed,² account for many of the discrepancies in seedling emergence between tests.

It has been a standard practice to pretreat unthreshed seed with sodium hypochlorite. Essentially the treatment (1) consists of soaking the seed in water for 18 to 20 hours and then treating the seed for 2 hours with a solution of sodium hypochlorite containing 1.0 to 1.5 per cent available chlorine, after which the seed is thoroughly washed, dried, and stored. The purpose of the hypochlorite treatment is to increase the germinable percentage of recently harvested guayule seed. Benedict and Robinson (3) found that the hypochlorite treatment accomplished this primarily by increasing the permeability of the seed coat which surrounds the embryo. While the hypochlorite treatment (pretreatment) is not applied for its fungicidal action, it effectively eliminates surface seed-borne organisms. Since both hypochlorite-treated and untreated seed may be treated with seed protectants (fungicides), the hypochlorite treatment is referred to as a pretreatment. Threshed seed may also be pretreated with sodium hypochlorite, although its practical value is questionable. Benedict and Robinson (3) have shown that either threshing or aging in the open, or both, will improve seed germination. They conclude "that in so far as germination is concerned the very expensive (sodium hypochlorite) seed treatment now given guayule seed can be eliminated by using achenes alone that have been stored in the open for six months or by producing seed in (hot) climates such as that at Indio, California, and holding it for a year before planting."

FUNGICIDES

A number of seed protectants were tested for pre-emergence damping-off control on both threshed and unthreshed guayule seed which had or had not

² By germinable seed is meant those seed which will germinate readily, in 10 to 14 days, under usual seed laboratory or greenhouse conditions.

been pretreated with sodium hypochlorite. The fungicides tested for pre-emergence damping-off control were Arasan (50 per cent tetramethyl thiuramdisulfide), Fermate (70 per cent ferric dimethyl dithiocarbamate), Cuprocid (98 per cent red cuprous oxide), Spergon (98 per cent tetrachloro-para-benzoquinone), Spergonex (*o*-benzoquinone dionium peroxide), Semesan (30 per cent hydroxymercurichlorophenol), U.S.R. No. 604 (2,3-dichloro-1,4-naphthoquinone), and Mersolite-19 (phenyl-mercuric-salicylate).

Of the various fungicides used, all have been reported on as seed protectants by various workers at one time or another on crops other than guayule with the exception of Mersolite-19 (9). Since reports on their efficacy may be found readily in the literature, no specific citations are given.

The fungicides were applied as a dust to dry seed. The most commonly used fungicidal dosage was 1 per cent by weight. The seed to be treated or dusted and the desired amount of fungicide were placed in an airtight container, either a small jar or a rotary seed treater depending on the amount of seed to be treated, and thoroughly mixed by shaking or rotating the container for approximately 5 minutes. When 300 seeds or less were to be sown in a single flat or row the seeds were counted and placed in a coin envelope after the fungicide was applied. The usual practice was to sow within 3 or 4 days after treatment. However, delays of 10 to 14 days or longer did not reduce seedling emergence.

GREENHOUSE TESTS

Unless otherwise indicated, 200 seeds were sown in each replication. The seeds were sown on soil in flats and covered with about $\frac{1}{2}$ inch of pasteurized sand. The sand was kept moist by frequent watering in order to secure maximum emergence. Seedling emergence was counted daily. The effect of the various seed treatments is based upon the total number of seedlings that emerged by the 15th day.

In most of the emergence tests in the greenhouse both pasteurized and fungus-infested soil were used. Damping-off fungi were eliminated from raw soil by means of a continuous soil pasteurizer (10). In the 6 minutes required for passage through the pasteurizer the soil was heated to 200° to 210° F., a temperature considerably higher than the 150° F. used by Tavernetti (10) to control damping-off caused by species of *Pythium* and *Rhizoctonia*. Fungus-infested soil was obtained by infesting pasteurized soil with various isolates of *Pythium* and *Rhizoctonia*, which had been isolated from nursery damped-off guayule seedlings.

Effective Fungicidal Dosage

Preliminary tests were made to determine, within limits, the fungicidal dosage required to give adequate pre-emergence damping-off control in fungus-infested soil. In general, the most effective dosage for Arasan, Semesan, Spergon, Spergonex, and Cuprocid was 1 per cent when used on either threshed seed or on unthreshed seed which had been pretreated with

TABLE 1.—Effect of 7 different fungicides on guayule seedling emergence

Sort of seed	Soil treatment	Fungicides and seedling emergence, 15 days after sowing							Difference required for significance
		Mersolite-19	No. 604	Arsan	Fermate	Spergon	Semesan	Spergonex	
		Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Threshed	Pasteurized ^b	136.6	149.2	140.4	136.8	140.6	141.6	98.2	112.8
	Infested	136.7	134.7	141.4	76.0	22.0	39.1	5.2	11.5
	Nursery	139.3	134.6	119.3	37.0	15.6	16.2	4.4	3.8
Unthreshed	Pasteurized	61.1	52.7	60.3	56.1	54.6	57.7	50.8	49.8
	Infested	56.9	52.3	54.4	21.2	10.3	39.8	3.0	2.6
	Nursery	55.5	45.4	50.8	14.7	7.0	21.4	1.5	1.3
Unthreshed, pretreated with hypochlorite	Pasteurized	37.7	91.7	113.7	107.9	105.9	77.8	99.1	94.0
	Infested	63.5	97.9	103.0	104.6	101.3	104.0	104.8	56.1
	Nursery	54.6	101.4	114.5	103.2	107.0	90.7	83.5	40.4

^a The seed treatment dosage for Mersolite-19 was 0.25 per cent; for No. 604 it was 0.75 per cent, and it was 1 per cent in the other treatments.
^b Only 5 replications of 200 seeds for each seed treatment; 10 replications in all other tests.

sodium hypochlorite. No apparent injury was caused by any of these 5 fungicides, at dosages of 1 per cent or less, to the germinating seed and emerging seedlings in either pasteurized soil or soil infested with *Pythium* and *Rhizoctonia*.

Seedling emergence was better in both pasteurized and fungus-infested soil when the seeds were treated with a 0.25 per cent dosage of No. 604 than with 0.5 or 1 per cent dosages of the same protectant. In the case of the pasteurized soil this difference was large enough to be significant indicating the possibility of chemical injury from dosages of 0.5 per cent or higher. However, this indication of injury was partially offset by the results from the fungus-infested soil where the difference in emergence between the 0.25 and 0.5 per cent dosages was not significant. On unthreshed hypochlorite-treated seed the emergence resulting from the 3 different dosages did not differ greatly.

Comparative Seed Treatment Tests

Three greenhouse tests (Table 1) were made to compare the effectiveness of 7 different fungicides in the control of pre-emergence damping-off when used on either threshed or unthreshed seed, or on unthreshed sodium hypochlorite-treated seed. The fungicidal dosages used were 0.25 per cent for Mersolite-19, 0.75 per cent for No. 604, and 1 per cent for Arasan, Spergon, Semesan, and Spergonex. There were 10 replications of each treatment in pasteurized soil, in soil which had been infested with cultures of *Pythium* and *Rhizoctonia*, and in raw or nontreated soil from the Alisal Nursery. Damping-off fungi were prevalent in the nursery soil. On the basis of isolations from affected seedlings the most prevalent was *Pythium ultimum*.

In nursery and inoculated soil infested with *Pythium* and *Rhizoctonia* the increase in emergence from seed treated with Arasan, No. 604, and Mersolite-19 was significantly greater than for any of the other treatments on both threshed (Fig. 1) and unthreshed seed which had not been pretreated with sodium hypochlorite (Table 1). On unthreshed seed which had been pretreated with sodium hypochlorite all of the fungicidal treatments increased emergence about equally except Mersolite-19 which gave little or no increase in emergence over the checks. With the exception of Mersolite-19 on hypochlorite-treated seed, there was little or no indication that the fungicides at the dosages used in these 3 tests caused any chemical injury. In all probability the 0.25 per cent dosage of Mersolite-19 was excessive when dusted on hypochlorite-treated seed and the chemical injury which resulted tended to offset its protective value.

The effects of the different fungicides on the rate of emergence when dusted on both threshed seed and hypochlorite-treated unthreshed seed sown in fungus-infested soil are given in figure 1. Seedling emergence was well under way by the 4th day after sowing for most of the treatments and continued at a rapid rate until about the 7th or 8th day when it began to level off.

Seedling emergence tests were made to determine if the standard sodium

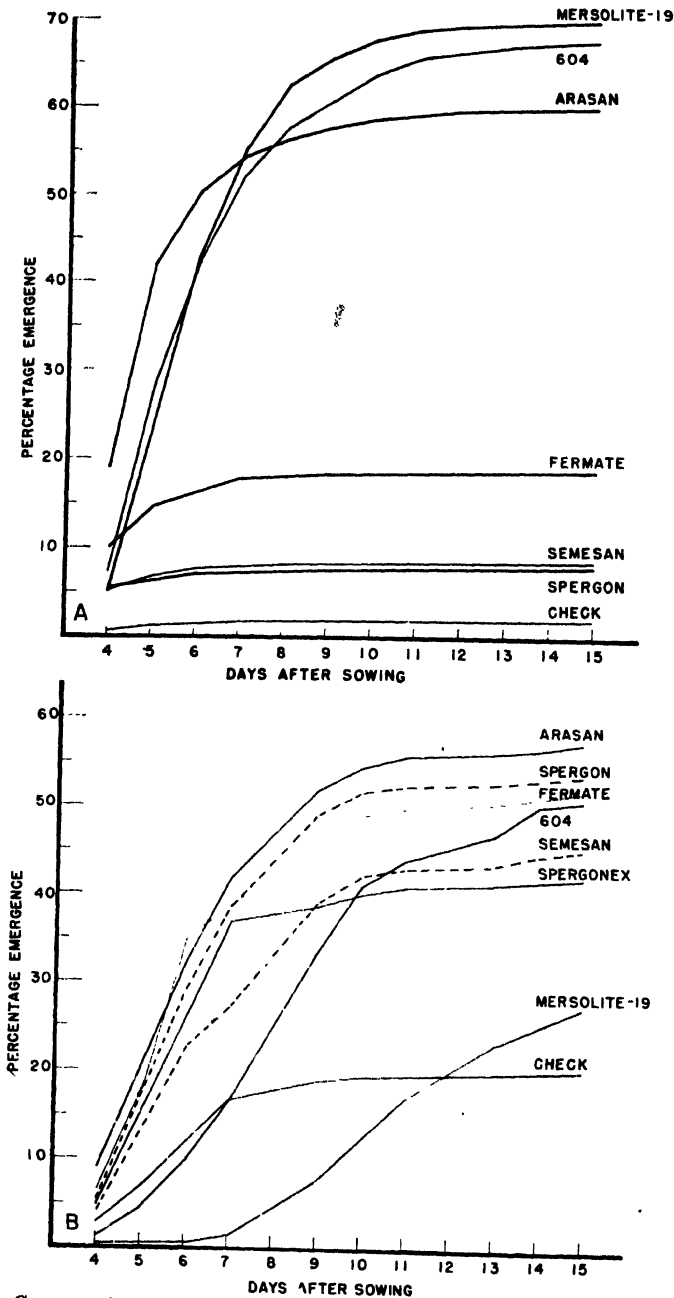


FIG. 1. Comparative effectiveness of fungicidal seed treatments and rate of guayule seedling emergence in nursery soil infested with damping-off fungi, mostly *Pythium ultimum*. A. Threshed seed. The emergence curve for Spergonex was practically the same as that of the check and for this reason was omitted. B. Unthreshed seed pretreated with sodium hypochlorite. Note the difference in response to the different fungicides on the two kinds of seed.

hypochlorite pretreatment would give effective pre-emergence protection against damping-off fungi and to compare its protective value with that of Arasan and No. 604. The results indicated that the sodium hypochlorite treatment did give some apparent protection against pre-emergence losses but that the increase in emergence was considerably less than that secured by the use of either Arasan or No. 604. Seedling emergence in fungus-infested soil was significantly better for threshed seed which had been treated with Arasan and No. 604 than for threshed seed which had received only the hypochlorite treatment. However, with hypochlorite-treated seed damping-off control was better when the seed was dusted with Arasan than with No. 604. On the other hand, in pasteurized soil the dusting of threshed hypochlorite-treated seed with No. 604 caused a significant decrease in emergence. This reduction in emergence was attributed to chemical injury. Sodium hypochlorite-treated seed appeared to be more sensitive to chemical injury than the raw seed. In this series of tests, as well as others, seedling emergence of threshed nonhypochlorite-treated seed in pasteurized soil was increased by dusting the seed with either Arasan or No. 604 (Table 1). The increases were so pronounced in some instances as to suggest the possibility of a stimulative action.

NURSERY TESTS

Three seed protectants, Arasan, Spergon, and Semesan, which were procurable on the local market, were used in two trial nursery sowings in the Alisal Nursery in the spring of 1944. The treated seed were sown in plots of sufficient size to permit the use of regular nursery equipment. The seed was sown and covered with about one-fourth inch of sand by a seeder developed especially for the sowing of guayule seed. All cultural operations were comparable to standard nursery practices (1). The amount of water applied by the overhead sprinkler system and frequency of application were similar to those in the nurseries in 1943.

Seed used in the nursery tests were: (1) dry threshed seed which had not been pretreated with sodium hypochlorite; (2) dry unthreshed seed which had been pretreated with sodium hypochlorite and (3) soaked or pregerminated seed which had received the regular sodium hypochlorite pretreatment. The fungicides, dosage 1 per cent by weight, were applied as dust to the dry seed by means of a small rotary drum-type seed treater. In case of the soaked seed, the seed was first dusted with the fungicide and then moistened with tap water. Twice as much water by weight was used as seed. The dampened seed was thoroughly mixed and placed in open-top 5-gallon cans in the laboratory where it was kept for 4 or 5 days before sowing. During this soaking period the seed was stirred twice for aeration.

Only one kind of seed, threshed, dry unthreshed, or soaked unthreshed, was sown in a nursery section composed of 9 or 10 nursery beds 400 feet long. Each bed was divided into 4 equal plots, 100 feet long, and was treated as a randomized block which included each seed treatment. The first sowing was on April 30 and the second on May 20.

The daily average temperature during the critical period of 10 days following sowing ranged from about 55° to 60° F. For the same period the maximum temperature exceeded 78° F. on two days only and the minimum temperature did not fall much below 50° F. These temperatures were favorable, but somewhat lower than the optimum, for germination and emergence as indicated by greenhouse tests in which 70° to 80° proved to be more favorable for emergence than 65° or lower. Also, Benedict and Robinson (3) in laboratory tests found that optimum germination of guayule seed occurred at 68° to 77° F.

Emergence counts were made 2 or 3 times a week for 4 weeks following sowing in "count" plots which had been established at random before any seedlings had emerged. These plots consisted of a linear foot section of bed, 4 sq. ft. with 4 to a replication. In order to secure as accurate measure as possible of emergence, the seedlings were pulled out at time of counting.

Results of Treatment

All three fungicidal seed treatments of dry threshed seed increased emergence over checks in both the April and May sowings (Table 2). In only one case was the increase not statistically significant at the end of 30 days, and this was near the borderline of significance. The emergence of threshed seed dusted with Arasan was better than for the other treatments. In the April (Table 2) sowing 79 per cent of the germinable seed dusted with Arasan had emerged by the 30th day after sowing, and 87 per cent of the viable seed in the May sowing.

In the May 20 sowing of dry unthreshed seed pretreated with sodium hypochlorite all treatments gave an increase in emergence over the check, but only Spergon gave a significant increase (Table 2). There was no significant difference in emergence between treatments at the end of 30 days in the April 30 sowing. No chemical injury was apparent with any of the treatments.

In the April 30 sowing, fungicidal seed treatments of soaked unthreshed seed pretreated with sodium hypochlorite did not significantly increase emergence (Table 2) over the control. In the May 20 sowing a significant increase in emergence was secured with both Arasan and Spergon. Seedling emergence from seed dusted with Semesan was sparse and scattered in both the April and May sowings and averaged less than 5 seedlings per square foot. Because of this failure, emergence counts of Semesan-treated seed are omitted from table 2. The deleterious action of Semesan apparently occurred during the 4-day period the seed was held in a moist condition prior to sowing.

The rate of emergence was somewhat more rapid in the April sowing than in the May sowing. In both cases emergence levelled off 18 to 20 days after sowing following a rather pronounced break about the 12th day. The difference in emergence between the two sowings, when based on viable seed sown, was not great enough to be considered important.

The inevitable loss which follows emergence occurred with all of the treatments in both the April and May sowings. There were no indications that the fungicidal seed treatments were effective in reducing post emergence losses. These losses (based on total emergence) ranged from 10 to 20 per cent for the different treatments at the end of 4 weeks after sowing. In a seedling density count made 8 weeks after sowing, the average loss of emerged seedlings had increased from about 15 per cent at the end of 4 weeks

TABLE 2.—*Effect of Arasan, Spergon, and Semesan on guayule seedling emergence in the nursery*

Seed treatment	Germinable seed sown per sq. ft.		Emerg'd seedlings per sq. ft.—30 days after sowing			
			Sown April 30 ^a		Sown May 20 ^b	
	April 30	May 20	Total	Percentage of germinable seed	Total	Percentage of germinable seed
	Number	Number	Number	Per cent	Number	Per cent
Dry threshed seed						
Arasan	76	68	60.37	79.43	59.29	87.19
Spergon	76	68	46.33	60.96	43.96	64.65
Semesan	76	68	44.59	58.67	40.16	59.06
Check	76	68	40.51	53.30	19.33	28.43
Dif. req. for sig.			5.13		6.74	
Dry unthreshed hypochlorite-treated seed						
Arasan	81	91	40.05	49.44	59.31	65.18
Spergon	81	91	42.54	52.52	62.74	68.95
Semesan	81	91	44.05	54.38	60.45	66.43
Check	81	91	43.07	53.17	54.09	59.44
Dif. req. for sig.			3.23		7.99	
Soaked unthreshed hypochlorite-treated seed						
Arasan	89	73	39.53	44.42	40.21	55.08
Spergon	89	73	49.64	55.78	38.15	52.26
Semesan	89	73				
Check	89	73	49.62	55.75	31.85	43.63
Dif. req. for sig.			5.86		4.75	

^a Ten replications of each treatment.

^b Nine replications of each treatment.

to approximately 25 per cent 4 weeks later. These losses were caused by post-emergence damping-off, seedling root rot, soil splash, weeding, and insects. No attempt was made to evaluate the losses attributable to any one cause because of the difficulty involved in making accurate determinations.

FIELD TESTS

The feasibility of guayule production by direct field sowing, as well as the production of nursery stock by furrow irrigation, has emphasized the importance of obtaining satisfactory seedling emergence and uniformity of stand under conditions different from that found in the nursery. Since

protectants were of benefit in increasing seedling emergence in the nursery and in the greenhouse, it was expected that similar results would be obtainable in subirrigated field sowings. However, it was realized that, while the conditions were similar in many respects in nursery and field sowings, there were also differences. In common nursery practice the seed is sown in beds, covered with sand and watered by an overhead sprinkler system, while in the field the seed is drilled in rows, covered with soil, and furrow irrigated. Because of these differences in sowing and watering it seemed probable that the germinating seed and emerging seedling would be subjected to greater pre-emergence hazards in field sowings than in the nursery.

Subirrigation plot tests were made during the summer of 1944 to ascertain the effectiveness of seed protectants in direct field sowings. Dry seed, either threshed or unthreshed, from two seed lots of strain 593, either 35-G or B-38, collected in 1943 were used. Only seed which had not been pre-treated with sodium hypochlorite was treated with fungicides but the standard sodium hypochlorite treatment was included as one of the treatments. The check or control received no chemical treatment.

The seed was sown with a Planet Jr. seeder in an open V-type furrow 1 to 1½ inches deep and 2 to 4 inches from the edge of a shallow irrigation furrow. An effort was made to cover the seed with a minimum amount of soil. The rear press wheel was removed from the seeder and the seed was covered only with the soil that incidentally rolled or drifted onto the seed from the sides of the furrow. In this manner the seed was sown at the bottom of the furrow on firm or fairly compact soil and covered with ¼ to ½ inch of loose soil in 30-foot row plots.

The plots were furrow irrigated every other day for 2 to 3 hours. However, this schedule was sometimes varied, depending upon weather conditions and obvious need of irrigation. An effort was made to keep the seed sufficiently moist for optimum germination and emergence rather than to follow a predetermined irrigation schedule.

Frequent emergence counts were made starting with the earliest emergence and continuing up to 14 or 15 days after sowing. The center 15 feet of the 30-foot plots were used for emergence counts.

The air and soil surface temperatures were generally favorable for germination and emergence during July and August. The average air temperature during the two months was around 60° F., except for a few days in the third week of August when it was slightly lower. The soil temperature at a depth 0.5 to 1.0 inch averaged 10° F. higher than the air temperature.

Results

In this series of experiments both the Arasan and No. 604 treatments increased emergence significantly over the hypochlorite treatment in both threshed and unthreshed seed (Table 3). Emergence in the check was uniformly poor in all cases. Seed dusted with No. 604 gave a significantly higher emergence than threshed seed treated with Arasan. On unthreshed

TABLE 3.—*Effect of fungicidal seed treatments on guayule seedling emergence in furrow irrigation tests*^a

Treatment	Emergent seedlings				
	Threshed seed			Unthreshed seed	
	July 13 ^b	July 13	Aug. 2	July 13	Aug. 2
	<i>Per cent</i> ^c	<i>Per cent</i> ^c	<i>Per cent</i> ^c	<i>Per cent</i> ^c	<i>Per cent</i> ^c
Arasan	19.36	25.29	26.21	36.15	57.29
No. 604	34.10	35.67	50.60	42.43	66.51
Hypochlorite	6.94	9.96	12.18	13.03	34.78
Check	2.40	0.92	3.60	0.47	1.29
Difference required for significance (5 per cent)	8.22	8.20	7.91	13.49	8.63

^a Emergence is based upon all seedlings which had emerged within 15 days after sowing in the middle 15 feet of a 30-foot row plot.

^b Date sown. Two different lots of threshed seed were sown on July 13; 35-G and B-38.

^c Percentage of germinable seed sown.

seed the emergence was higher for the No. 604 treatment, although it was significantly so in only one of two instances.

SUMMARY AND CONCLUSIONS

Guayule (*Parthenium argentatum* Gray) was found to be very susceptible to attack by pre-emergence damping-off fungi in nurseries and in direct field sowings at Salinas, California. Because of pre-emergence losses extensive resowings of nursery beds were necessary in 1943. Post-emergence damping-off losses were small compared with pre-emergence losses.

In greenhouse tests with 8 fungicides to determine their relative effectiveness in protecting guayule seedlings against pre-emergence losses from *Pythium* and *Rhizoctonia*, damping-off fungi, the optimum dosage was about 1 per cent by weight for Arasan, Spergon, Spergonex, Cuprocide, and Seme-san; 0.5 to 0.75 per cent for No. 604; and 0.25 per cent or less for Mersolite-19.

Treating guayule seed with sodium hypochlorite increased seedling emergence in soil infested with *Pythium* and *Rhizoctonia* in the greenhouse as well as in sub-irrigated field-sown tests. However, when seed which germinated readily was dusted with Arasan or No. 604, emergence was higher than from hypochlorite-treated seed when sown in fungus-infested soil. The differences in protective effect were significantly in favor of the seed protectants over sodium hypochlorite.

When used on both threshed and unthreshed non-hypochlorite-treated seed in the greenhouse Arasan, No. 604, and Mersolite-19 gave the greatest increases in seedling emergence in fungus-infested soil. On unthreshed seed, which had been pretreated with sodium hypochlorite, Mersolite-19 did not increase emergence as much as Arasan and No. 604.

Under conditions favorable for germination in the greenhouse, guayule seedlings started to emerge on the 4th day and emergence was practically completed in 10 to 12 days after sowing.

On dry, threshed, non-hypochlorite-treated seed sown in the nursery, Arasan, Spergon, and Semesan increased emergence over the untreated seed in sowings made in April and May. With threshed guayule seed the treatment with Arasan resulted in more emerged seedlings than either Spergon or Semesan treatment.

Dry unthreshed seed that had been pretreated with sodium hypochlorite, when dusted with Arasan, Spergon, or Semesan gave an increase in emergence over the check in a sowing made in May. In an April sowing the differences in emergence for the seed dusted with these seed-protectants were not significant. Seed similarly treated, but soaked for 4 days prior to sowing, gave no increased emergence for any of the materials in the April sowing. In the May sowing a significant increase in emergence was secured with both Arasan and Spergon. In both sowings the emergence from seed dusted with Semesan was sparse, scattered, and considerably lower than for the check.

In a subirrigated field both Arasan and No. 604 increased seedling emergence significantly above the hypochlorite treatment and above non-hypochlorite-treated checks for both threshed and unthreshed seed. Emergence of threshed seed dusted with No. 604 was better than when Arasan was used.

Based on tests in greenhouse, nursery, and subirrigated field Arasan and No. 604 are considered as the two most promising of the several seed protectants tested in the control of pre-emergence damping-off of guayule. Although tested only in the greenhouse, Mersolite-19 gave excellent results.

SPECIAL GUAYULE RESEARCH PROJECT,

BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL
ENGINEERING,

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BREEDING FOR RESISTANCE TO EARLY BLIGHT IN THE IRISH POTATO¹

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Early blight, caused by *Alternaria solani* (Ellis and G. Martin), L. R. Jones and Grout, does not usually occur in epiphytotic form, although occasionally it may be almost as destructive as late blight. Coons⁴ stated that the annual loss in Michigan was about 25 per cent. According to Jones,⁵ the yield may be reduced 10 to 25 per cent in Wisconsin. Several cases are on record of unusually severe attacks; more important, however, is the smaller but yearly toll of the disease.

No concerted effort has been made to breed new varieties of potatoes that are resistant to the pathogen causing early blight, although it has been known for many years that varieties of cultivated potatoes have different degrees of susceptibility. The early work of Stuart⁶ in Vermont indicated that a few foreign varieties possessed some resistance to the fungus.

In 1940 work was started toward finding resistant seedlings or commercial varieties that could be used in a breeding program. The data herein are presented as a preliminary report.

METHODS AND MATERIALS

Conditions favorable for a natural epiphytotic were not present during the growing seasons of 1940 to 1943, inclusive. The laying of a pipe line in 1943 permitted the use of supplemental water as an overhead spray on experimental plots to make conditions more favorable for infection and subsequent spread of the fungus. By the spring of 1945 methods had been developed so that a heavy infection was produced and it was possible to ascertain the resistance or susceptibility of a number of seedlings and varieties.

In this experiment the seedlings and varieties were grown adjacent to a row of the Houma variety, which is very susceptible to the organism.

The infection data were recorded in 4 classes as illustrated in figure 1:

Class 0 = No infection.

Class 1 = Light infection; spots few to many, small, not coalescing; foliage remained green.

¹ Cooperative investigations by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture and the Louisiana Agricultural Experiment Station.

² Pathologist; U. S. Department of Agriculture, Baton Rouge, Louisiana.

³ The writer takes pleasure in acknowledging his indebtedness to Dr. F. J. Stevenson for furnishing many of the seedlings and many of the parents used in crosses that were included in this investigation.

⁴ Coons, G. H. Potato diseases of Michigan. Mich. Agr. Exp. Sta. Special Bull. 66. 1914.

⁵ Jones, L. R. Potato diseases in Wisconsin and their control. Wis. Agr. Exp. Sta. Circ. 36. 1912.

⁶ Stuart, William. Disease resistance of potatoes. Third report. Vt. Agr. Exp. Sta. Bull. 179: 147-183. 1914.

Class 2 = Moderate infection; spots few to many, medium to large, some coalescing; foliage 0-25 per cent killed.

Class 3 = Severe infection; spots many, medium to large, coalescing; foliage burned, 26-100 per cent killed.

Conditions very favorable for infection prevailed during all of May, 1945. By May 3 most of the leaves were killed on the Houma checks and many of the seedlings and some varieties were severely infected.

Because of differences in maturity of the material being tested, notes were taken on several dates.

In this test, 19 named varieties and 445 numbered seedlings were exposed to infection. Each of these was grown in units of at least 5 hills. The seedlings represented many different crosses and a few inbred parents. Since no segregating progenies were included, it is not possible to draw conclusions as to the mode of inheritance of resistance to the early-blight fungus.

RESULTS

The reactions of 19 commercial varieties of potatoes to early blight are in table 1. All but 2 of these varieties were very susceptible to the fungus. Of the 2, Sequoia was moderately infected, but the infection in Menominee was only slight. Menominee also possesses a high degree of resistance to *Actinomyces scabies* and moderate resistance to *Phytophthora infestans*. The early-maturing varieties—Cobbler, Earlane, Red Warba, Triumph, and Warba—were severely infected early in May whereas some of the later-maturing sorts were not infected to the same degree until 2 weeks later. The data also demonstrate that lateness is not completely correlated with

TABLE 1.—Progressive development on 19 commercial varieties of Irish potatoes of infection by the early-blight fungus in the field at Baton Rouge, Louisiana, in 1945

Variety	Incidence of disease* on			
	May 3	May 5	May 18	June 2
Chippewa	0	3	3	3
Cobbler	3	3	3	3
Desoto				3
Earlane	3	3	3	3
Earlane No. 2	0	0	2	3
Golden	0	0	3	3
Green Mountain	0	3	3	3
Kasota	0	3	3	3
Katahdin	0	0	3	3
LaSalle				3
Menominee	0	0	1	1
Mesaba	3	3	3	3
Mohawk	0	3	3	3
Pontiac	3	3	3	3
Red Warba	3	3	3	3
Sebago	0	0	3	3
Sequoia	0	0	2	2+
Triumph	3	3	3	3
Warba	3	3	3	3

* Infection classes; 0 = no infection, 1 = light, 2 = moderate, 3 = severe.

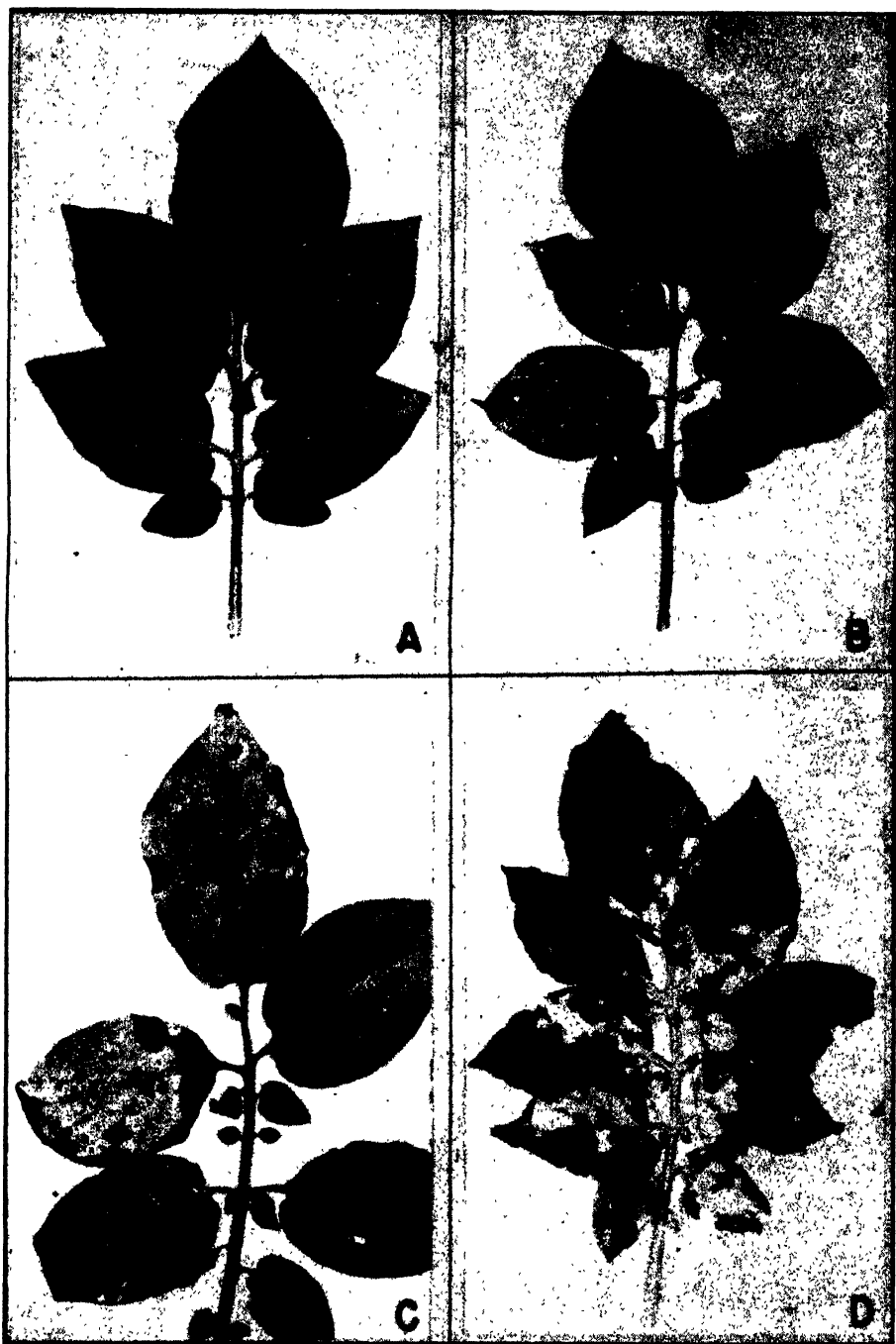


FIG. 1. Infection classes: A = no infection (class 0); B = light infection (class 1); C = moderate infection (class 2); D = severe infection (class 3).

early-blight resistance since the late-maturing and very-late-maturing varieties, such as Earleine No. 2, Katahdin, Mohawk, and Pontiac, were killed by the fungus before they matured.

The 20 seedlings listed in table 2 were selected to represent the various groups of infection types that were found among the 445 seedling varieties tested. Seedlings XL 127-2 and XL 154-7 were severely infected early in May and both of these are early-maturing sorts. Infection was delayed in many other seedlings listed in table 2.

TABLE 2.—*Progressive development on some seedling varieties of Irish potatoes of infection by the early-blight fungus in the field at Baton Rouge, Louisiana, in 1945*

Pedigree	Parentage	Incidence of disease* on			
		May 3	May 5	May 18	June 2
XL 127-2	96-140 × 926-36	3	3	3	3
XL 154-7	47562 × 47156	3	3	3	3
XL 28-1	336-123 × 336-18	0	0	2	3
XL 29-23	Katahdin × Earleine	0	0	2	2
XL 133-9	Sebago × 47156	0	0	1	3
XL 133-6	Sebago × 47156	0	0	1	2-
X 512-1	W1-6 × Katahdin	0	0	1	2-
X 987-1	792-78 × Katahdin	0	0	1	2-
B 94-3	336-144 × 47562	0	0	1	2-
B 70-5	B 127 × 96-56				3
XL 148-1	528-229 × 528-170				1+
XL 8-61	Chippewa × 47156				1
XL 211-1	(XL 89-1) × (XL 72-1)				1
XL 306-2	499-a × B 56-11				1
XL 308-5	Pontiac × B 44-14				1
X 528-118	Richter's Jubel × 44537				1
X 627-103	Hindenberg × Katahdin				1
B 69-12	Katahdin × 96-56				1
B 273-31	President × 96-28				1
B 273-39	President × 96-28				1

* Infection classes; 0 = no infection, 1 = light, 2 = moderate, 3 = severe.

From the standpoint of breeding for resistance to the early-blight pathogen it is significant that a number of seedlings were found that were only slightly infected. Several of these are listed in table 2. Four of these seedlings (XL 211-1, XL 306-2, X 528-118, and X 627-103) are moderately to highly resistant to the scab organism. A very high degree of resistance to the late-blight fungus occurs in B 273-31 and B 273-39. Seedlings XL 148-1, XL 8-61, and B 69-12 are very vigorous and produce high yields of tubers. Seedling XL 308-5 is red-skinned and vigorous.

SUMMARY AND CONCLUSIONS

A large-scale, field test was made in 1945 at Baton Rouge, Louisiana, to test the reactions of 19 named varieties and 445 seedling varieties to the early-blight fungus (*Alternaria solani*).

The results failed to demonstrate the presence of immunity. A number of the seedlings were only slightly affected whereas the greater part of them

were moderately to severely affected. Of 19 named varieties tested, only *Menominee* had any marked degree of resistance.

Among the seedling varieties only slightly affected by the *Alternaria*, 4 are also moderately to highly resistant to the scab organism, 2 possess a high degree of resistance to the late-blight fungus, 3 are very vigorous and produce high yields of tubers, and one is red-skinned and vigorous.

Thus, from the standpoint of breeding for resistance to early blight a number of promising seedling varieties are at hand that can be used in future pollination work. In addition, some of these seedlings also possess genetic factors for resistance to the pathogens causing scab and late blight as well as factors for yielding ability. Hence it should not be very difficult to produce varieties combining resistance to early blight with resistance to other diseases as well as with other characters of commercial importance.

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LEAFROLL NET NECROSIS AND STEM-END BROWNING OF POTATO TUBERS IN RELATION TO TEMPERATURE AND CERTAIN OTHER FACTORS

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INTRODUCTION

A crop from a potato (*Solanum tuberosum* L.) field has no potentiality for the development, in storage, of leafroll net necrosis unless at least three conditions prevail. First, the variety must be susceptible to the leafroll virus. Second, leafroll must have infected plants, during the growing season, that were healthy at the beginning of the season. Third, the variety must be one that can develop net necrosis as a symptom of recent infection by leafroll. However, even when having the potentiality resulting from the fulfillment of these three conditions, crops, parts of crops, and tubers have been observed which either did not develop net necrosis in storage or developed much less than was to be expected. Another type of internal discoloration, called stem-end browning, was found commonly in the Maine potato crops of 1923, 1927, 1929, and 1937 and was differentiated from net necrosis (3, 4). This paper is concerned with the effects of temperature, length of storage period, size of tuber, and certain other factors, upon the development of net necrosis and stem-end browning in storage.

In 1940 the cause of stem-end browning was still undetermined and attempts were made, as they had been previously, to isolate organisms from affected tubers. Isolations were unsuccessful in the majority of cases, and none of the miscellaneous lot of organisms obtained produced any stem-end browning or other change when inoculated into healthy tubers (10, p. 503).¹ In the winter of 1942-43, steam sterilization of soil from fields that had produced stem-end browning crops did not reduce stem-end browning in the greenhouse crop of plants grown in the sterilized soil, in comparison with greenhouse crops in unsterilized soil from the same fields (13, p. 232). About 180 hills were dug individually in 1943 in 6 fields. Field notes were taken on stem infection by *Rhizoctonia solani* and, later, storage records were taken on the development of stem-end browning at about optimum temperature. In general "very slight" *Rhizoctonia* infection was followed by the highest percentages of stem-end browning, but the most stem-end browning was in the tubers from "healthy" hills in the case of one field, and in the tubers from "severely infected" hills in the case of another field.

Recently it has been determined by Ross (14, p. 530-531) that stem-end browning in Maine can become, under suitable storage conditions, much more prevalent in one strain of the Green Mountain variety than it can in other strains of the same variety in the same environment. Ross (14, p.

¹ Data are given in considerable detail in the Maine Agricultural Experiment Station report bulletins referred to in this paper.

529-530) has further transmitted this potentiality from one tuber line or plant to another by grafting. His data indicate that a virus is one factor in the development of stem-end browning.

Small-scale preliminary tests with Green Mountain tubers of the crop of 1937 disclosed a tendency for more internal discoloration to develop at 35°, 46°, and 53° F. than at 71°, 86°, or 96°, at constant temperature, or than at a variable temperature of 32° to 64°, averaging 43° (9, p. 800). Similarly, a series of Green Mountain samples of the 1938 crop developed net necrosis in 0, 11, 13, 0, 0.1, and 0 per cent of the tubers when kept at 40°, 42°, 56°, 76°, 83°, and 70° to 90° F. respectively (9, p. 800). Another 1938 series at these same temperatures developed respectively 0, 1.9, 1.3, 0.3, 0.4, and 0 per cent net necrosis and 0, 1.9, 3.2, 0.7, 0.7, and 0 per cent stem-end browning (9, p. 800).

These preliminary tests indicated an optimum constant-temperature range of about 42° to 56° F. for both net necrosis and stem-end browning. They also indicated that such temperature tests require many identical samples of two to three bushels each.

More samples of larger size, composed to be identical, were taken from the crop of 1939 and stored in seven places, with results as follows:

Temperature, in °F.	32	37	41	45	54	72	77	86
Net necrosis, in per cent	0	2	3	1	4	1	0	0
Stem-end browning, in per cent	1	11	9	6	9	2	1	0

Here the 45° F. storage had a fluctuating temperature that, with an average near optimum as judged by results in constant-temperature storages, was nevertheless less conducive than they were to the development of internal discoloration, just as was the case in the 1937 crop. Apparently it is the number of hours near optimum temperature that counts, more than the average temperature.

METHODS WITH CROPS OF 1940 TO 1945

Twelve experimental storage bins each with a capacity of about 100 barrels (a "barrel" being 165 lb.) were constructed at Aroostook Farm, near Presque Isle, Maine, in the summer of 1940 (10, p. 498). These bins were equipped with cooling apparatus, heaters, portable calcium chloride driers, and sprinkling apparatus for the maintenance of temperatures from 30° F. up and for the partial control of humidity. Samples of a crop were shifted from one bin to another if a change of temperature or humidity was desired. All samples of any crop were made up and examined by the same operator.²

Unless stated to be otherwise, samples were of the Green Mountain variety and were made up in sets of 36 from each field as follows. Thirty-six field-run barrels of tubers were spread out, a barrel at a time. From each barrel the 36 apparently largest tubers were distributed to the 36 samples. This was repeated until the contents of the barrel were completely

² Frank Upton, in crops of 1940 and 1941, and Michael Goven, in crops of 1942, 1943, and 1945.

divided. Each barrel was taken in turn so that the end result was 36 composite samples each containing 1/36 of each original field-run barrel and all practically identical as to tuber sizes. When duplicates were kept in the same bin for the same length of time, the percentage of tubers with net necrosis or stem-end browning was practically the same, so that each series of 36 samples could be used for comparing 36 different combinations of temperature, duration, and humidity. No method was found successful for making more than one examination of a given sample.

EFFECTS OF TEMPERATURE AND RELATIVE HUMIDITY

Results with the Crop of 1940

Tests were made with 432 samples (12 series of 36 each) from the crop of 1940 from four fields. There was the least of either kind of internal discoloration at 32–33° F., the most at 50°, and intermediate percentages at 38° and 60°. Several comparisons by Student's method were possible, each between 22 to 24 samples in one bin and the same number in another bin, the members of each pair coming from the same series of 36 composite samples made up at harvest time. The results, given in table 1, show in comparisons 1, 4, and 5 that 5 to 12 degrees' difference gave highly significant differences in the percentage of tubers with net necrosis or stem-end browning; in the other comparisons, with little or no difference in temperature there were only nonsignificant differences in internal discoloration. The

TABLE 1.—*Effect of temperature differences on percentage of tubers having net necrosis and developing stem-end browning in storage. Samples compared by Student's method.^a Crop of 1940*

Com- parison	Pairs ^b	Tempera- ture	Relative humidity	Net necrosis		Stem end browning	
				Means	Odds ^c	Means	Odds ^c
		°F.	Per cent	Per cent		Per cent	
1	22	33	86	3.33	128: 1	1.14	61: 1
		38	89	6.61		1.75	
2	22	38	77	4.88	14: 1	1.44	Under 10: 1
		38	89	6.61		1.75	
3	22	39	79	8.42	3.67: 1	2.40	Under 2: 1
		38	89	7.81		2.32	
4	24	38	89	5.36	Over 9999: 1	2.12	Over 9999: 1
		50	88	10.60		13.29	
5	22	39	79	8.42	Over 3332: 1	2.40	Over 9999: 1
		50	82	17.67		14.12	
6	22	50	75	17.27	Under 3: 1	13.44	Under 10: 1
		50	82	17.67		14.12	
7	23	60	71	6.78	Under 10.5: 1	10.99	Under 6.52: 1
		60	79	6.10		10.39	

^a Method as given in (8).

^b In each comparison, 22 to 24 samples, of 700 to 1000 tubers apiece, held in one storage locker were paired respectively with the same number of samples in another storage locker, the members of each pair having come from the same series of 36 composite samples and having been stored for the same length of time.

^c Odds considered significant only if 30 or more to 1.

relative humidity (71 to 89 per cent) had no apparent effect on either kind of defect. At 50° F., stem-end browning continued to increase appreciably until the middle of November, and net necrosis usually until in December or January but until in March or April in some series of samples. Samples kept at low temperatures until in November and then warmed to the optimum temperature developed net necrosis but not stem-end browning, indicating a permanent inhibition of stem-end browning by exposure to low temperature during the first part of the storage season.

Results with the Crop of 1941

No attempt was made to control humidity after the tests with the crop of 1940, although relative humidity was recorded daily. The 288 samples (8 series of 36 each) of the crop of 1941 from 4 fields were variously exposed to temperatures of 33°, 37°, 40°, 44°, 47°, 51°, and 60° F. The optimum tested temperatures for the development of net necrosis were 44°, 47°, and 51° F., respectively, for the three series having an appreciable amount of the defect, running about 33, 28, and 37 per cent (11, p. 298-302, 329). In the case of stem-end browning the percentage of affected tubers was greater at 60° F. (running from 9 to 47 per cent) than at lower temperatures. The maximum percentage was reached or nearly reached in 45 to 65 days after harvesting.

Some samples were shifted from suboptimum to optimum temperatures. Samples held 10 to 30 days at 33° F. and then moved to 47° developed less net necrosis than samples held from the first at 47° in the same period of time. Further, 30 to 90 days at 33° apparently conditioned the tubers so that subsequent exposure to 47° was followed by no further development, or very little, of net necrosis. In one series of samples, stem-end browning developed in 120 days in 13 per cent of the tubers stored at 33°, in 33 per cent at 51°, in 14 per cent at 33° for 60 days followed by 51° for 60 days, and in 23 per cent at 33° for 30 days followed by 51° for 90 days. Apparently 60 days at 33° F. "conditioned" the tubers so that subsequent exposure to 51° was followed by practically no further development of stem-end browning.

Results with the Crop of 1942

One hundred forty-four samples (4 series of 36 each) from four fields, crop of 1942, were variously exposed to storage temperatures of 31°, 33°, 36°, and 51° F. at which the relative humidity averaged, respectively, about 76, 75, 78, and 78 per cent. The maximum percentage of tubers with net necrosis was reached, or practically reached, at 52° F., in 60 to 90 days after digging, and, with stem-end browning, in 60 to 120 days after digging. Samples kept until 200 days after digging showed, in some series, some decrease from the maximum in percentage net necrosis or with stem-end browning at both 51° and 36° F. (13, p. 279). The data in table 2 show that considerably more net necrosis and stem-end browning developed in 90 days in storage at 51° F. than at 31°, 33°, or 36°. The difference was

somewhat less in the samples from Field 3 where about 12 per cent of the tubers already had net necrosis at harvest time.

Some samples were exposed first to suboptimal or supraoptimal temperatures and then to about optimum temperature. The data, in table 3, show that 60 days' "conditioning" at 31°, 33°, 36°, or 71° F. reduced considerably the amount of net necrosis or stem-end browning that developed subsequently in 60 days at 51° F., in stocks where the discoloration potential was present, as compared with the development in 60 to 120 days at a constant temperature of 51°. The permanently inhibiting effect of extreme temperatures was somewhat less in the samples from Field 3 where net necrosis had already developed in 12 per cent of the tubers by harvest time. In the other series of samples the suppressing effect of low temperature was

TABLE 2.—*Development of net necrosis and stem-end browning in samples of 1942 crop kept for 90 days at several constant temperatures*

Field	Net-necrosis percentage				Stem-end browning percentage			
	31° F.	33° F.	36° F.	51° F.	31° F.	33° F.	36° F.	51° F.
1	0	0	0	0.4	0.6	0.7	1.7	21.7
2	0	0	0	0.1	1.1	1.3	1.6	39.5
3	15.3	17.1	20.0	36.2	1.4	1.5	1.5	6.4
3 ^a	3.4	5.2	8.1	24.3				
4	0.4	0.6	0.7	15.0	0.7	0.7	0.4	15.9

^a Increase over the 11.9 per cent at harvest time, when storage at different temperatures was begun.

somewhat greater on stem-end browning than on net necrosis, and high temperature inhibited net necrosis somewhat more than low temperature did.

Results with the Crop of 1943

One hundred forty-four samples (4 series of 36 each) from four fields, crop of 1943, were variously exposed to storage temperatures of 32°, 36°, 70°, 65°, and 50° F. at which the relative humidity averaged, respectively, about 79, 78, 78, 78, and 87 per cent. At 50°, the maximum percentage of tubers with net necrosis was reached in 90 to 100 days and the percentage then gradually declined until in April when the tubers were too rotted, sprouted, and shrivelled for accurate diagnosis. At 50°, the maximum percentage of tubers with stem-end browning was not reached in two series until 120 to 240 days after harvest time, with no subsequent decline. Some samples, listed in table 4, showed that conditioning at 70° or 32° F. for 60 days, previous to 60 days at 50° F., completely prevented or greatly reduced the development of net necrosis and stem-end browning. Conditioning at 65° or 36° was less effective in some instances. Other samples in the same series (13, p. 278) showed that at 70° and 65° shorter periods of conditioning (30 and 15 days) were often much less effective than was conditioning for 60 days.

Field 2 had been in potatoes the past three years, was on the same farm as Field 2 of 1941 and Field 1 of 1942 in these studies, and was expected to

yield a crop with as much stem-end browning as these other fields had. The drop to practically none can be attributed to a change in the source of seed,

TABLE 3.—*Net necrosis and stem-end browning in samples of 1943 crop and the permanently inhibiting effect of low or high temperature during first part of storage*

Field	Days in storage ^a at					Net necrosis	Stem-end browning
	31° F.	33° F.	36° F.	71° F.	51° F.		
						<i>Per cent</i>	<i>Per cent</i>
1					60	0.3	16.2
	60				60	0.0	0.4
		60			60	0.0	0.4
			60		60	0.0	0.9
					120	0.3	22.1
2					60	0.2	30.6
	60				60	0.0	0.3
		60			60	0.1	0.5
			60		60	0.0	1.2
					120	1.7	42.1
3					60	29.7	5.2
	60				60	18.7	2.2
		60			60	17.1	1.6
			60		60	26.6	3.3
					120	35.3	5.7
3 ^b					60	17.8	
	60				60	6.8	
		60			60	5.2	
			60		60	14.7	
					120	23.4	
4					60	15.8	11.1
	60				60	1.0	0.0
		60			60	2.2	0.4
			60		60	4.7	1.5
					120	17.1	14.7
1				60	30	0.4	8.2
					30	0.1	1.0
					90	0.4	21.7
2				60	30	0.0	23.3
					30	0.0	0.9
					90	0.1	39.5
3				60	30	22.7	4.8
					30	12.2	2.1
					90	36.2	6.4
3 ^b				60	30	10.8	
					30	0.3	
					90	24.3	
4				60	30	9.4	5.1
					30	1.9	1.8
					90	15.0	15.9

^a Periods in storage at different temperatures given from left to right as they occurred in the experiment.

^b Increase over the 11.9 per cent at harvest time, when storage at different temperatures was begun.

the owner of the farm having been convinced that there was a difference between strains of Green Mountain with respect to the ability to develop stem-end browning.

TABLE 4.—*Net necrosis and stem-end browning in samples of 1943 crop and the permanently inhibiting effect of low or high temperature during first part of storage*

Field	Days in storage ^a at					Net necrosis	Stem-end browning
	32° F.	36° F.	70° F.	65° F.	50° F.		
						<i>Per cent</i>	<i>Per cent</i>
1	60	60	60	30	30	61	0.0
						61	0.3
						61	0.0
						61	0.2
						61	0.0
						61	8.6
						61	34.6
						60	25.5
3	60	60	60	30	60	121	30.0
						61	0.1
						61	0.0
						61	0.0
						61	0.6
						61	0.1
						61	2.8
						61	0.0
						61	7.8
						60	5.5
						60	18.6
						121	6.1
							4.5

^a Periods in storage at different temperatures given from left to right as they occurred in the experiment.

Results with the Crop of 1915

No study was made of these defects with the 1944 crop. However, 72 samples (2 series of 36 each) from the crop of 1945, from two fields, were exposed to storage temperatures of 34°, 38°, 44°, and 50° F. at which the relative humidity averaged, respectively, about 83, 83, 85, and 90 per cent. At 50° F. the maximum percentage of tubers with net necrosis was reached in 70 to 90 days and with stem-end browning in 100 to 130 days. Each kind of internal discoloration was graded in each tuber as either "cull" or "mild," the distinction being made on the basis of whether more or less than 5 per cent of the weight of the tuber had to be sliced off in order to remove all discoloration. This is a distinction used in the U. S. grades for potatoes, grade No. 1 having tolerance for only a certain amount of tubers with 5 per cent waste as determined by slicing. The several temperatures were considered to be representative of potato storages (2, Tables 1 and 5) and certain samples were shifted from suboptimum to optimum temperatures to simulate the succession of storage, transit, and market conditions in commercial practice.

As indicated in table 5, exposure to 50° F. for one or two weeks after exposure to a low temperature often resulted in considerably more net necrosis than developed in the same period during exposure to only the low temperature. For example, in sample 215 after 72 days at 34° F. there was only 3 per cent cull net necrosis while in sample 214 after 56 days at 34° and 15 days at 50° (total 71 days) there was 7 per cent cull net necrosis. However, a shift from 34° or 38° to 50° did not have a similar increasing effect upon stem-end browning, which apparently was permanently inhibited by these two lower temperatures more than was net necrosis. The

TABLE 5.—*Effect of storage temperature on net necrosis and stem-end browning in samples of 1945 crop*

Sample ^a	Days in storage ^b at					Av. temp.	Tubers with			
	34° F.	38° F.	44° F.	50° F.	All temp.		Net necrosis		Stem-end browning	
							Cull ^c	Total	Cull ^c	Total
						°F.	Per cent	Per cent	Per cent	Per cent
102	28			8	36	38	1	2	0	T ^d
105		28		7	35	40	3	5	0	0
108			28	7	35	45	8	10	0	T
111				35	35	50	10	13	1	6
104	43				43	34	1	2	0	0
103	28			14	42	39	1	5	0	0
107		43			43	38	2	3	T	T
106		28		14	42	42	3	7	0	0
110			43		43	44	8	10	0	T
109			28	14	42	46	7	10	0	1
112				42	42	50	10	17	2	6
113	56			8	64	36	1	3	0	0
116		56		8	64	40	3	5	0	0
119			56	8	64	45	8	10	1	5
122				64	64	50	11	12	10	30
115	70				70	34	1	3	0	0
114	56			14	70	37	1	5	0	0
118		71			71	38	4	8	0	0
117		56		14	70	40	3	7	0	0
121			71		71	44	9	11	1	2
120			56	14	70	45	8	9	1	3
123				70	70	50	10	12	9	23
124	84			7	91	35	1	2	0	0
127		84		7	91	39	3	6	0	0
130			84	7	91	45	9	10	1	6
133				91	91	50	10	10	14	32
126	100				100	34	1	3	0	0
125	84			14	98	36	0	3	0	0
129		99			99	38	4	6	0	0
128		84		14	98	40	4	6	0	0
132			99		99	44	9	10	2	9
131			84	14	98	45	9	9	3	10
134				98	98	50	10	10	16	36
135				99	99	50	10	10	15	34
136				126	126	50	8	8	17	34
202	28			9	37	38	4	8	0	0
205		28		9	37	41	7	13	0	0
208			28	10	38	46	23	26	0	0
211				37	37	50	23	26	T	T
204	43				43	34	2	5	0	0
203	28			15	43	40	4	10	0	0
207		44			44	38	5	9	0	0
206		28		15	43	42	11	16	0	0
210			44		44	44	22	24	0	0
209			28	16	44	46	22	25	0	0
212				43	43	50	28	32	1	1
213	56			8	64	36	7	16	0	0
216		56		8	64	40	12	16	0	0
219			56	8	64	45	23	26	T	1
222				64	64	50	31	32	2	3

TABLE 5 (Continued)

Sample ^a	Days in storage ^b at					Av. temp.	Tubers with			
	34° F.	38° F.	44° F.	50° F.	All temp.		Net necrosis		Stem-end browning	
							Culls	Total	Culls	Total
						°F.	Per cent	Per cent	Per cent	Per cent
215	72				72	34	3	5	0	0
214	56			15	71	37	7	14	0	0
218		72			72	38	7	12	0	0
217		56		15	71	41	14	18	0	0
221			72		72	44	26	28	1	1
220			56	15	71	45	31	32	1	1
223				71	71	50	31	32	1	2
224	86			8	94	35	7	14	0	0
227		86		8	94	39	15	21	0	0
230			86	8	94	45	29	31	1	1
233				93	93	50	33	34	2	2
226	100				100	34	4	9	0	0
225	86			15	101	36	12	20	0	0
229		102			102	38	10	16	0	0
228		86		17	103	40	20	25	0	0
232			103		103	44	27	29	1	1
231			86	15	101	45	28	31	1	1
234				102	102	50	32	33	2	2
235				103	103	50	31	33	2	3
236				128	128	50	32	32	4	4

^a Samples 102 *etc.* from Field 1, 202 *etc.* from Field 2.

^b Periods in storage at different temperatures given from left to right as they occurred in the experiment.

^c Cull net necrosis or stem-end browning is that which causes more than 5 per cent sliced-off waste.

^d "T" means "trace" or less than 0.5 per cent.

results given in table 5 were used to determine correlation between the average storage temperature and the percentage of cull net necrosis and of total net necrosis. The correlation coefficients are respectively +0.845 and +0.852 for the samples from Field 1 and +0.893 and +0.879 for the samples from Field 2.³ The relationship may be expressed by equations for linear regression as follows:

Field 1, cull net-necrosis percentage = 0.57 (average ° F.—32.7° F.)
 Field 2, cull net-necrosis percentage = 1.73 (average ° F.—32.1° F.)
 Field 3, cull net-necrosis percentage = 0.56 (average ° F.—29.0° F.)
 Field 4, cull net-necrosis percentage = 1.47 (average ° F.—27.8° F.)

This means, for example, that for every degree of temperature over 32.7° F., the samples from Field 1 developed 0.57 per cent cull net necrosis. Therefore the proportion of tubers developing either cull net necrosis or all degrees of net necrosis was determined largely by the excess of average temperature over a given temperature that varied, with the four sets of determinations, from 27.8° to 32.7° F.

³ Highly significant (18, Table 16).

TABLE 6.—*Rate of development of net necrosis and stem-end browning in crops of 1940 to 1945, in storage at or near optimum temperature*

Crop year	Field and series	Date of harvest	Tubers with rot necrosis			Tubers with stem-end browning			Tubers per sample
			At harvest	Maximum	Date of maximum	At harvest	Maximum	Date of maximum	
			Per cent	Per cent		Per cent	Per cent		
1940	1-B			59	Apr. 25		11	Jan. 9	Over 800
	1-C ^a			33	Dec. 12		6	Nov. 22	Over 700
	2-B			20	Jan. 27		7	Dec. 19	Over 800
	2-C			15	Mar. 4		7	Dec. 2	Over 800
	3-B			8	Apr. 26		20	Jan. 28	Over 900
	3-C			8	Mar. 5		17	Jan. 16	Over 900
	4-B			T ^b	Apr. 29		32	Apr. 29	Over 1000
	4-C			1	Oct. 21		36	Dec. 16	Over 1000
1941	1-A	Sept. 22	7	42	Dec. 23		10	Jan. 22	Over 600
	1-B	Sept. 22	6	41	Nov. 26	2	13	Dec. 27	Over 600
	2-A	Sept. 30	0	1	Dec. 30	4	39 ^c	Jan. 30	Over 600
	2-B	Sept. 30	0	1	Jan. 2	5	53 ^c	Nov. 18	Over 600
	3-A	Sept. 29	4	31	Feb. 4	0	5	Feb. 4	Over 500
	3-B	Sept. 29	4	24	Feb. 6	0	13	Feb. 6	Over 500
	4-A	Oct. 6	10	34	Nov. 18	1	6	Mar. 10	Over 600
	4-B	Oct. 6	10	38	Nov. 19	1	11	Feb. 17	Over 600
	1	Sept. 17	0	T	Oct. 19	0	22	Dec. 17	About 700
	2	Sept. 23	0	2	Jan. 23	T	42	Jan. 23	About 800
	3	Sept. 12	12	36	Dec. 12	0	6	Dec. 12	About 500
	4	Sept. 30	T	17	Jan. 28	0	16	Dec. 30	About 700
1942	1	Sept. 20	0	4	Dec. 29	1	41	May 16	About 400
	2	Oct. 13	0	1	Dec. 2	1	2	Feb. 10	About 400
	3	Sept. 25	3	26	Dec. 14	T	10	Oct. 15	About 400
	4	Oct. 4	2	20	Jan. 4	1	13	Oct. 4	Over 500
1945	1	Sept. 20	T	12	Nov. 29	0	36	Dec. 27	About 600
	2	Oct. 6	1	34	Jan. 7	0	4	Feb. 11	About 700

^a From part of field treated differently from other parts sampled.^b "T" means "trace" or less than 0.5 per cent.^c The maximum was 39 per cent at 47° F. and 53 per cent at 60° F.

There is not a similar relationship between temperature and stem-end browning in this test, because the lower temperatures had a greater inhibiting effect upon this kind of discoloration than upon net necrosis.

EFFECT OF TIME OF EXAMINATION

All internal discoloration had developed by December 15 in the 1937 crop. In the 1938 crop, the maximum of net necrosis was reached by November 23 and December 27, respectively, in two series, but of stem-end browning not before February 11 (time of last examination of any samples). In the 1939 crop, the maximum net necrosis was reached in February and the maximum stem-end browning by mid-December. Similar data for the composite samples of later crops are given in table 6, and show that there usually was some net necrosis and stem-end browning at harvest. Harvest dates occurred from September 12 to October 13. Most of either kind of dis-

TABLE 7.—*Net necrosis and stem-end browning in duplicate samples in the same series*

Crop	Temperature	Period	Field	Sample	Special treatment	Net necrosis	Stem-end browning
	°F.	Days				Per cent	Per cent
1942	52	150	1	A		0.1	19.6
				B		0.6	19.7
			2	A		1.2	41.0
				B		0.6	37.8
			3	A		35.5	6.2
				B		36.1	6.1
			4	A		15.1	15.2
				B		15.3	14.4
1943	50	120	1	A		0.3	24.6
				B		1.1	30.7
				C	Clipping ^a	1.8	4.3
				D	Splitting ^b	1.9	5.3
				E	Do	0.4	5.1
			2	A		0.3	2.0
				B		0.5	2.3
				C	Clipping	0.0	0.0
				D	Splitting	0.0	0.0
				E	Do	0.0	0.0
			3	A		20.9	4.9
				B		20.5	4.7
				C	Clipping	10.1	0.3
				D	Splitting	6.1	0.0
				E	Do	4.1	1.0
			4	A		19.6	8.4
				B		18.1	8.0
				C	Clipping	14.0	0.0
				D	Splitting	10.4	0.0
				E	Do	14.6	0.0
1945	50	100	1	A		9.8	35.8
				B		10.1	34.1
			2	A		33.3	2.0
				B		33.4	2.6

^a Each tuber had $\frac{1}{8}$ inch of the stem-end clipped off at the beginning of storage.

^b Each tuber was split at the beginning of storage, half going into sample D and half into sample E.

coloration developed in storage. Net necrosis reached a maximum by October 19 to April 29, usually by the end of January. Stem-end browning reached a maximum by October 4 to May 16, usually by the end of February.

DUPLICATES

Comparisons 2, 6, and 7 of table 1 show that, in the 1940 crop, duplicate samples in the same series developed practically the same percentages of tubers affected with net necrosis or stem-end browning if kept for the same length of time at the same temperature, even if in different lockers.

Duplicate samples of the crop of 1942 were kept at 52° F. for 150 days. The results of examination, given in table 7, show very close agreement between duplicates.

Duplicate samples of the crop of 1943 were kept at 50° F. for 120 days. The results, given in table 7, show very close agreement between nontreated duplicates in most cases. Duplicates with the stem-end clipped off for $\frac{1}{8}$ inch at the beginning of storage had net necrosis usually reduced and stem-end browning eliminated or greatly reduced. Splitting of the tubers at the beginning of storage to make two split-tuber duplicate samples had the same reducing effect as clipping, and the split-tuber duplicates often agreed with one another less than the whole-tuber duplicates in the same series.

Duplicate samples of the crop of 1945, kept at 50° F. for 100 days, also agreed closely one with another (Table 7).

Altogether, these results showed that the composite samples made as described were replicates very similar as to potentiality for the development of net necrosis or stem-end browning. Repeated examination of the same tubers apparently does not yield reliable data.

EFFECT OF SIZE OF TUBER

Using the crop of 1937, Folsom and Rich (4, p. 321) showed that stem-end browning increased in frequency as tuber weight decreased, while net necrosis, where abundant, decreased in frequency.

When the 72 samples from the crop of 1945 were examined, in each instance the record of net necrosis and stem-end browning was made by centuries of tubers, always examining in turn the 100 apparently largest as a group until the sample was finished. From the records thus obtained, the average was determined for the 8 or 9 centuries of largest tubers stored entirely or predominantly at 34° F., then for the next largest tubers, and so on; this was repeated for temperatures of 38°, 44°, and 50° F.

The results, in table 8, show that in each field, at every storage temperature, the percentage of tubers developing net necrosis, whether cull, mild, or total, tended to decrease with smaller tuber size with few exceptions. For example, the part of the crop from Field 2 kept at 50° F. averaged 46.3 per cent net necrosis for the largest-tuber centuries and decreased, as relative tuber size decreased, to 41.5, 37.1, 33.0, 23.6, 21.4, and 17.9 per cent, respectively, for the centuries with progressively smaller tubers. One pos-

TABLE 8.—*Effect of tuber size on net necrosis and stem-end browning. Crop of 1945*

Field	Tempera- ture ^a	Century ^b	Tubers with net necrosis ^c			Tubers with stem-end browning			Ratio cull per- centage to total percentage	
			Cull ^d	Mild ^e	All	Cull ^d	Mild ^e	All	N.N.	S.E.B.
	°F.		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent		
1 ^f	34	1	1.0	5.1	6.1	0	0	0	0.164	..
		2	0.8	2.8	3.6	0	0	0	0.222
		3	0.9	2.3	3.2	0	0	0	0.281
		4	0.7	1.6	2.2	0	0	0	0.318
		5	0.8	1.0	1.8	0	0	0	0.444
		6	0.9	0.6	1.4	0	0	0	0.643
	38	1	4.2	7.0	11.2	0	0	0	0.375
		2	3.2	3.6	6.8	0	0	0	0.471
		3	3.2	3.0	6.2	0	0	0	0.516
		4	3.2	1.8	5.0	0	0	0	0.640
		5	2.7	1.2	3.9	0	0	0	0.692
		6	1.8	1.1	2.9	0	0	0	0.621
	44	1	12.6	2.0	14.6	0	2.0	2.0	0.863	0.000
		2	11.1	2.4	13.6	0.2	2.6	2.8	0.816	0.071
		3	8.7	1.9	10.6	0.3	4.6	4.9	0.821	0.061
		4	8.0	1.3	9.3	1.2	5.1	6.3	0.860	0.190
		5	4.7	1.2	5.9	1.6	3.1	4.7	0.797	0.340
		6	4.4	0.4	4.9	2.1	1.6	3.7	0.898	0.568
	50	1	16.6	1.9	18.5	1.3	21.3	22.5	0.897	0.058
		2	12.0	1.1	13.1	3.8	23.5	27.3	0.916	0.139
		3	9.4	0.8	10.1	10.0	18.3	28.3	0.931	0.353
		4	9.9	1.6	11.5	13.9	14.5	28.4	0.861	0.489
		5	6.1	1.8	7.9	16.5	8.1	24.6	0.772	0.671
		6	4.9	1.4	6.3	15.5	3.3	18.8	0.778	0.824
2 ^g	34	1	7.4	14.7	22.1	0	0	0	0.335	
		2	6.6	9.2	15.8	0	0	0	0.418	
		3	6.9	7.0	13.9	0	0	0	0.496	
		4	5.3	4.1	9.5	0	0	0	0.558	
		5	4.3	2.1	6.4	0	0	0	0.672	
		6	5.1	0.9	6.0	0	0	0	0.850	
		7	3.1	0.2	3.3	0	0	0	0.939	
	38	1	18.2	12.6	30.8	0	0	0	0.591	
		2	13.0	8.3	21.3	0	0	0	0.610	
		3	12.8	5.6	18.3	0	0	0	0.699	
		4	11.0	3.4	14.4	0	0	0	0.764	
		5	9.3	2.1	11.4	0	0	0	0.816	
		6	8.1	1.2	9.3	0	0	0	0.871	
		7	6.3	0.2	6.6	0	0	0	0.955	
	44	1	37.4	5.0	42.4	0.1	0.1	0.2	0.882	0.500
		2	33.3	4.1	37.4	0.1	0.1	0.2	0.890	0.500
		3	25.6	3.4	29.0	0.6	0.1	0.7	0.883	0.857
		4	25.8	1.4	27.2	0.6	0.1	0.7	0.949	0.857
		5	21.7	1.2	22.9	0.9	0.2	1.1	0.948	0.818
		6	19.3	0.8	20.1	1.0	0.1	1.1	0.960	0.909
		7	16.0	0.2	16.2	0.8	0	0.8	0.988	1.000
	50	1	42.5	3.8	46.3	0.1	0.4	0.5	0.918	0.000
		2	39.5	2.0	41.5	0.8	0.3	1.0	0.952	0.800
		3	35.0	2.1	37.1	1.5	0.3	1.8	0.943	0.833
		4	31.4	1.6	33.0	2.0	0.3	2.3	0.952	0.870
		5	23.0	0.6	23.6	2.4	0.1	2.5	0.975	0.960
		6	20.3	1.1	21.4	3.5	0.4	3.9	0.949	0.897
		7	17.5	0.4	17.9	2.9	0	2.9	0.978	1.000

^a Temperature at which all or most of the storage period of 35 to 128 days was spent.

^b Each 100 tubers were selected as the largest appearing ones in the sample. Hence those in the first century were the largest, those in the second were the next largest, etc.

^c Each figure represents 800 or 900 tubers, 100 from each of 8 or 9 samples.

^d Cull net necrosis or stem-end browning is that which causes more than 5 per cent sliced-off waste.

^e Mild net necrosis or stem-end browning is that which causes less than 5 per cent sliced-off waste.

^f With seed-originated leafroll in 5 per cent of the hills.

^g With seed-originated leafroll in 12 per cent of the hills.

sible explanation of this positive correlation between relative tuber size and percentage of tubers with net necrosis is that there was a corresponding correlation between relative tuber size and new leafroll infection. Another is that with the same incidence of leafroll infection there was a tendency for larger tubers to react more often than smaller ones by developing net necrosis. Finally, both may occur.

The results with respect to stem-end browning were somewhat similar in the 44° and 50° F. series of samples from Field 2, in cull and total percentages. Here the percentage tended to increase with smaller tuber size. In Field 1 there was no correlation between tuber size and total percentage. The cull percentage increased and the mild percentage decreased with smaller tuber size, presumably because stem-end browning was shallow in all tubers and caused 5 per cent waste more often in smaller tubers.

An increase in the ratio of cull percentage to total percentage with a decrease in tuber size, was true (Table 8, last two columns) generally of both kinds of discoloration. It was less pronounced with respect to net necrosis in samples held at 44° and 50° F., probably because these temperatures were optimum not only for percentage of tubers developing the symptom but also for penetration deep enough to cause 5 per cent waste even in most of the larger tubers.

RATIO OF NET NECROSIS TO NEW LEAFROLL INFECTION

Net necrosis appears in late summer or fall before harvesting, or in storage. It appears only in tubers that have been invaded by the leafroll virus during the growing season in which they were produced. Such tubers may be called potentially net-necrosis tubers. The proportion of these that actually develop net necrosis is a ratio that sometimes varies with tuber size.

Using data of Schultz and Folsom (16, p. 70) on the crop of 1919, the following relation between tuber weight and percentage of newly infected leafroll tubers showing net necrosis is calculable for three seed stocks:

Tuber weight, in oz.	2	2-3	2-4	2-5	2-6	2-7	2-8	2-9	2-10	2-11
NN/LR, in per cent, lot A	54	59	65	66	67	68	69	71	72	
B	67	67	75	71	75	72	72	72	72	67
C	0	100	100	83	82	88	87	87	87	

In lot A there is a consistent increase in the ratio as the average tuber weight is greater. The same relation is evident from further data of Schultz and Folsom (17, p. 64) on the crop of 1921:

Tuber weight, in oz.	1-2	1-3	1-4	1-5	1-6	1-7	1-8
NN/LR, in per cent	45	59	64	67	71	76	79

The proportion of net necrosis to new leafroll infection probably varies also with variety, temperature, length of time elapsed after maturing of the tubers, time of plant inoculation by aphids,⁴ time of harvesting (11, p. 296),

⁴ The results of grafting by Bonde, Simpson, and Hovey (15) in the summer of 1944 were that the proportion of leafroll tubers that showed net necrosis rose from 0.08 in grafts made July 5 to 0.82 in grafts made August 9 and then declined to 0.53 in grafts made September 2. If experimental infection by aphids should show a similar correlation between time of infection and proportion of newly infected leafroll tubers that get net necrosis, it would be reasonable to suppose that the proportion referred to might vary from one field to another according to the time of the greatest infection by aphids.

degree of maturity at harvesting (10, p. 498), and kind and amount of mineral fertilizer applied (11, p. 297-298; 12, p. 432-433; 13, p. 218-219). It therefore should not be surprising to find that the NN/LR ratio varies considerably even when all the leafroll is of recent infection. Folsom and Rich (4, p. 315) found in the crop of 1937 an average ratio of about 1:3.3, with extremes of 1:1 and 1:9, among 29 Green Mountain seed stocks. In the 1939 crop grown on Highmoor Farm, near Monmouth in southwestern Maine, although half the tubers were newly infected with leafroll there was no net necrosis in samples stored at 32°, 37°, 41°, 45°, 54°, 72°, 77°, and 86° F.

In Aroostook-grown samples of the crop of 1942, the ratio of net necrosis percentage (at 51° F.) to new leafroll percentage varied considerably between different varieties and between different samples of Green Mountains. It was less than 0.01 for Chippewa, about 0.05 for Sequoia, about 0.10 for Katahdin, about 0.20 for Sebago, from about 0.20 to 0.40 for Irish Cobbler, and 0.27 to 1.00 (averaging 0.79) for 14 Green Mountain samples, as compared with 0.20 for Green Mountain grown on Highmoor Farm (12, p. 220 and 276).

EFFECT OF REGION

Stem-end browning was found in 28 of 30 bins examined in central Maine in the 1929 crop, with an average of 9 per cent of the tubers affected. The 1929 crop in Aroostook County in northeastern Maine had considerable net necrosis, but only one stored stock had an appreciable amount of stem-end browning. The 1937 crop in Aroostook County had still more net necrosis, but of 52 stored stocks examined, only 4 had more than 1 per cent of the tubers with stem-end browning.

At harvest time in 1942 in Aroostook County, soil temperature was found to be slightly below the optimum temperature for the development of net necrosis. One locker was held at the same temperature as that of the soil during harvest time, and samples kept in it developed almost as much net necrosis and stem-end browning as samples kept at 51° F.

In Pennsylvania⁵ although several types of stem-end browning were troublesome, leafroll net necrosis was seldom found.

In New York, on Long Island, Green Mountains are subject to considerable leafroll spread, but develop little if any net necrosis. The reason probably is the high soil temperature prevailing (over 70° F.) up to the time potatoes are harvested (1, p. 9).

Aphid transmission of leafroll to caged plants in Wisconsin in 1927 induced net necrosis evident on October 25, two months after harvesting, in 50 to 100 per cent of the tubers (6, p. 28-31). Net necrosis apparently was common in Vermont in 1926 (6, p. 10).

Considerable spread of leafroll occurred in Indiana in about 1922 but apparently without causing net necrosis (5) although the variety concerned has shown net necrosis in Maine.

⁵ Letter of April 25, 1940, from K. W. Lauer, Plant Pathologist of the Pennsylvania State Dept. of Agric., in charge of potato seed certification.

Generally it seems from the literature and from the temperature relations disclosed in this paper, that net necrosis can develop as a symptom of leaf-roll only in certain northern states. If a state or potato region is far enough South, soil, storage, and market are too warm for the development of net necrosis even if leafroll spreads readily and varieties are grown that easily develop the symptom at optimum temperatures. If a state or potato region is far enough North, leafroll is not common and does not spread often. There is a zone where leafroll spreads fairly often and where the temperature at the end of the growing season and at the beginning of the storage season favors the development of net necrosis; here either potato growers learn to grow a variety with practically no net-necrosis potentiality (such as Bliss Triumph, Katahdin, or Chippewa) or else take their losses from growing varieties (such as Green Mountain) that will develop the symptom when leafroll dissemination, optimum storage temperatures, and other factors favorable to the expression of this defect occur in the same season. After working in Maine storages (2), Edgar writes (letter of September 18, 1945) that "in the early part of storage the average temperatures in Maine storages will be 48°; the average to January 1 is usually 44° although the temperature January 1 may be below 40°." Therefore most storage temperatures in Maine may be considered generally about optimum for the development of net necrosis in Green Mountains under the prevailing conditions of well-distributed Chippewa and Green Mountain sources of leafroll dissemination.

INCREASE IN TRANSIT

Eight stocks of potatoes of the crop of 1939 were sampled by Cedric Porter, inspector for the Division of Markets, Maine State Department of Agriculture. From each stock three similar samples were taken. One was examined immediately, one was returned to the storage whence it came, and one was sent on with a carload of potatoes and was examined by one or another local inspector at the point of destination of the shipment (Washington, D. C., Pittsburgh, Cleveland, Baltimore, Philadelphia, Brooklyn, or Boston). The percentage of potatoes commercially defective^a from internal discoloration of the net-necrosis and stem-end browning types increased more in home storage than in transit in four out of six comparisons where there was an increase. In the other two comparisons it decreased less in one case. Therefore there was no evidence that shipment induced consistently either more or less increase than home storage.

However, in the case of stocks kept at minimum temperatures, there could be an increase of net necrosis and stem-end browning in transit and

^a "Commercially defective" means that a tuber loses over 5 per cent of its weight in the course of having all discolored tissue sliced off. The 5 per cent waste test disqualifies some small tubers because of stem-end browning, in spite of the discoloration being comparatively shallow. Since waste is determined by slices at any angle to give elimination of internal discoloration with the least loss of weight, some large tubers may have net necrosis without being disqualified by the 5 per cent test. Inspectors do not differentiate the two kinds of discoloration in their records and do not include all that there is of either kind in their records.

market, especially early in the season, due to change of temperature from too low to optimum. After low temperature has acted long enough on a stock to permanently inhibit further development of these internal discolorations even at optimum temperature, shipments from such stock may be characterized by much less internal discoloration than parallel shipments from other stocks not kept at a low enough temperature to prevent the development of internal discoloration within 90 to 120 days after harvest.

The results previously described for the samples of the crop of 1945 show that considerable increase in percentage of tubers with net necrosis can occur in a week or two at 50° F. following periods at lower temperatures. This could easily explain some differences between inspectors' reports at point of shipment and those at destination. However, such differences could be due in part to unreliable or insufficient sampling, bearing in mind especially the effect of tuber size.

CONTROL

Control of net necrosis or stem-end browning through the permanently inhibiting effect of an initial period at a low temperature probably would require artificial refrigeration of storages. The low temperatures required would prohibit keeping Chippewa or Katahdin potatoes in the same storage because of mahogany browning (7; 12, p. 291). Control of stem-end browning through the permanently inhibiting effect of an initial period at a high temperature such as 70° would require heating of storages within a very narrow range, inasmuch as 60° F. is not far from the optimum for development of the discoloration. Heat prevention might be practicable for the control of net necrosis. However, heating may be dangerous inasmuch as the percentage of tubers developing late blight has been increased from less than 5 per cent, at 32–36° F. for 60 days followed by 50° F. for 60 days, to over 20 per cent, at 65–70° F. for 15 to 60 days followed by 50° for 60 days (13, p. 288).

Knowledge of the optimum temperature and required period of time for the development of net necrosis and stem-end browning has helped indirectly toward control through improving tests of the effects of strain, variety, fertilizer, *etc.*, upon these discolorations.

The work reported in this paper indicates that the control of leafroll through selection of stocks showing little or no net necrosis in samples kept at optimum temperature, is not feasible because of the low ratio of net necrosis to new leafroll infection in some fields.

Reasonably good control of stem-end browning in the Green Mountain variety is possible through the use of seed stocks tracing back to certain sources in Vermont, Minnesota, and southwestern Maine (14, p. 530–531). An example was noted in discussing the temperature experiments with the crop of 1943.

The most desirable means for the control of net necrosis and stem-end browning probably would be commercially suitable new varieties immune from the causes of both.

DISCUSSION

The fact that early storage temperatures may determine largely the amount of net necrosis or stem-end browning, explains in part how differences can arise with respect to these forms of internal tuber discoloration in different storage houses or in different bins in the same storage house even when filled from the same field. Differences in elevation of storage bins, in ventilation practices, in insulation, or in the temperature of the tubers harvested at different times, can influence the temperature enough to affect the development of the maladies in question. Interseasonal differences also may be explained in part by differences in temperature at the time of maturity, harvest, and fall storage.

Interesting questions awaiting biochemical study are why storage temperature influences the development of net necrosis and stem-end browning, why clipping of the stem-end and tuber splitting reduce such development, why tuber size apparently sometimes influences this development, why leaf-roll infection induces net necrosis only when of recent occurrence, and why only part of such recent leafroll infection induces net necrosis even at optimum temperature.

SUMMARY

Leafroll net necrosis of potato tubers develops in Maine mostly during the first two or three months after harvesting. The percentage of incidence becomes highest at constant temperatures of about 45° to 50° F. and is very low at 33° and 70°. These threshold temperatures in 60 days can change potentially net-necrosis tubers so that they will not develop the discoloration later even upon being subjected to optimum temperatures. Greater tuber size is sometimes correlated with greater frequency of net necrosis and with a higher proportion of net-necrosis tubers among those recently invaded by the leafroll virus. Varieties that do not develop net necrosis as a leafroll symptom become relatively more desirable when the spread of leaf-roll increases in a region characterized by soil and storage temperatures that are about optimum for net necrosis. Net necrosis can sometimes increase considerably in transit and market in the event of a change in temperature toward the optimum. Various factors can influence storage temperature and so indirectly affect net necrosis. A number of field factors, but not relative humidity in storage, also can affect net necrosis.

Stem-end browning of potato tubers in Maine, not attributable to bacterial or fungus organisms, but apparently requiring a virus for its development, reacts to temperature and some other factors much as does net necrosis. However, the critical temperatures are somewhat higher, and smaller tuber size rather than larger increases the percentage of incidence.

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CONTROL OF VIRUS DISEASES OF CABBAGE SEED PLANTS IN WESTERN WASHINGTON BY PLANT BED ISOLATION

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INTRODUCTION

Prior to 1942 practically all of the cabbage seed produced in the United States was grown in the Puget Sound area, the greatest concentration of acreage being in Skagit County, Washington. The lower Skagit valley is relatively small, being less than 25 miles in either dimension. Since cabbage is a biennial crop, there is no crucifer-free period during which disease inoculum and insect pests can be completely eliminated as a source of virus inoculum. Seed is sown in large plant beds² in early June and the ensuing seed crop harvested during July and August of the following year. Thus there is an overlapping period of about two months during which seedling plants for a crop just started are exposed to certain diseases and insect pests affecting the seed crops approaching harvest.

It is a regular occurrence in this area for infestations of the cabbage aphid (*Brevicoryne brassicae* L.) to build up in seed fields during spring and to reach peak intensity during this two-month overlapping period. Winged forms migrate thence into young plant beds when the latter are in close proximity. Since the cabbage aphid is a vector of crucifer viruses,^{3, 4, 5} any such infection of seed plants would be readily transmitted to seedling plants. Such has actually been the case. In the seed crops harvested in 1943 and 1944 approximately 60 per cent of all seed plants were infected with cabbage mosaic.⁶ Of 6 large plant beds sown in June, 1943, for the 1944 crop, 2 were immediately adjacent to such seed fields and the other 4 were less than 100 rods removed. By actual counts in 2 of these large plant beds, approximately 80 per cent of the plants were infected with mosaic before they were transplanted. During the same season a small experimental plant bed grown by the Seed Laboratory approximately 30 miles from the seed-growing area had no evidence of virus infection.

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² The common practice is for a seed-contracting company to grow plants for its entire acreage in one large plant bed. These plants are distributed to growers at transplanting time.

³ Larson, R. H., and J. C. Walker. A mosaic disease of cabbage. *Jour. Agr. Res. [U.S.]* 59: 367-392. 1939.

⁴ Tompkins, C. M. A transmissible mosaic disease of cauliflower. *Jour. Agr. Res. [U.S.]* 55: 33-46. 1937.

⁵ Tompkins, C. M., M. W. Gardner, and H. Rex Thomas. Black Ring, A virus disease of cabbage and other crucifers. *Jour. Agr. Res. [U.S.]* 57: 929-943. 1938.

⁶ Walker, J. C., Francis LeBeau, and Glenn S. Pound. Viruses associated with cabbage mosaic. *Jour. Agr. Res. [U.S.]* 70: 379-404. 1945.

Beginning with the 1944-45 crop, it was recommended that seed companies isolate their plant beds, by several miles if possible, from such diseased fields. Consequently, 60 per cent of the plants transplanted in the fall of 1944 had been grown several miles from seed fields. The remaining 40 per cent of the plants were from plant beds located within the seed-growing area and near seed fields. For the 1945-46 crop over 90 per cent of the plants were from plant beds grown in isolated areas. It was thought that if healthy plants were used in the fall transplanting, the amount of mosaic in the seed fields would be materially reduced. Although it was surmised that aphid activity in the fall after transplanting would result in some infection, it was expected that the ultimate extent of infection would not be great. This hope was based on the failure to find turnip fields severely affected with mosaic. Turnip, which is very susceptible to mosaic, is sown in late fall after aphid activity is greatly reduced and thereby evidently escapes severe infections. This paper reports the effect of isolation of plant beds in reducing the amount of mosaic in cabbage seed fields.

METHODS

About 6 to 8 weeks after transplanting, a systematic disease survey was made of the cabbage acreage in both 1944 and 1945. At least 70 per cent of the acreage planted from each plant bed was included in the survey. In 1944 a total of 85 fields, comprising 957 acres, was checked. This represented almost 80 per cent of the entire planting in the Skagit area. In 1945 only 63 fields and 601 acres were surveyed. This was approximately 72 per cent of the entire acreage. The number of virus-infected plants was counted in three sample rows (one on each side and one in the middle of the field) of 200 plants each for each field visited. If a field was planted with plants from different sources, 600 plants from each source were checked and the ultimate percentage infection of the field determined by averaging the values, weighting them according to the proportion of the field planted from each source.

In order to determine the extent of infection after the fall survey, the acreage of 1944-45 crop was again surveyed in the late spring of 1945 when plants were in early bloom, using the method described. This survey included 55 fields and 720 acres. Another limited survey was made one month prior to harvest in which a few selected fields were checked. This last survey was to determine the extent of severity of infections occurring in late spring.

RESULTS

The 1944-45 Crop.—During the fall survey very great differences existed between plants from isolated plant beds and those from local, nonisolated plant beds. Plants from the isolated areas established themselves more quickly and were more vigorous when they entered the winter. Much of this difference in growth and vigor is attributed to the stunting of local-grown plants by the mosaic disease and to the relative freedom from this disease of plants from isolated areas.

Isolation of plant beds greatly reduced the amount of mosaic in transplanted fields in the fall (Table 1). Over 53 per cent of the plants from

TABLE 1.—*Effect of plant bed isolation on the amount of mosaic in cabbage seed fields, 1944-45*

Source of plants and season of survey	Percentage of plants infected	Percentage of fields with:			
		No infection	Over 15 per cent infection	Over 40 per cent infection	Over 60 per cent infection
Fields planted from isolated beds:					
Fall, 1944	0.8	42.0	0.0	0.0	0.0
Fields planted from isolated beds:					
Spring, 1945	3.0	2.9	2.9	0.0	0.0
Fields planted from local beds: ^a					
Fall, 1944	53.0	0.0	100.0	69.4	30.6
Fields planted from local beds: ^b					
Spring, 1945	55.1	0.0	100.0	84.2	42.2

^a Includes 2 large plant beds and several smaller ones. All were grown within 1 mile of a diseased seed field.

^b Includes only the 2 large plant beds referred to in footnote a.

local plant beds had mosaic symptoms and no field in which they were used was free of infection. Only 0.8 per cent of the plants from isolated plant beds had mosaic symptoms, and 42 per cent of the fields had no infection at all. These results are in spite of the fact that aphids occurred in plant beds in sufficient amount to cause widespread infection had they migrated from virus-infected plants. Table 2 shows the average percentage infection

TABLE 2.—*Relation of plant bed isolation to amount of mosaic in cabbage seed fields, 1944-45*

Plant bed	Degree of isolation from diseased seed fields	Average percentage of infected plants in transplanted fields
A	Excellent	0.23
B	Excellent	0.27
C	Excellent	0.49
D	Excellent	1.00
E	Poor	46.90
F	Very poor	72.60

of the acreage planted from individual plant beds. Beds A, B, C, and D were in well-isolated areas, the closest (C) being 7 miles from the nearest seed field, with dense woods separating them. The other 3 were 20 miles or more from seed fields. Bed E was in the seed-producing area but was about 200 rods from the nearest seed field. Bed F was also within the seed-producing area and was only 20 rods from diseased seed fields. It is believed that infection found in fields using plants from isolated areas was initiated for the most part after transplanting, since many fields from each of the beds had no infection. These data show that isolation of plant beds was sufficient to provide healthy plants at transplanting time.

Since aphids were found in many of the transplanted fields it was necessary to check the acreage again in late spring in order to determine the extent of infection after the fall survey. This survey was made in early May when plants were in early bloom. Since aphid infestations in this area are very light in early spring and do not reach severe proportions until late May or June, it was believed that such a survey would reveal the approximate extent of losses this disease would effect in seed yields. The incubation period of the viruses in seed plants is 30 days or more and infections initiated as late as June would not effect serious losses.

This late spring survey (Table 1) revealed that the acreage planted from isolated plant beds was still markedly free of mosaic when compared with that from local plant beds, the average infections being 3.0 and 55.1 per cent, respectively. There was an increase in the amount of mosaic of both acreages of slightly over 2 per cent. The highest infection in any field from isolated plant beds was 20 per cent. Without exception, the fields showing the highest increase were those of late-maturing varieties which were transplanted earliest in the fall and thus exposed longer to viruliferous aphids. The increase in the varieties transplanted later in the fall was much less. There was also an apparent relation between the percentage increase of mosaic and the amount of aphids present in the fields in the fall.

Some fields provided excellent tests of whether the beneficial effects of plant bed isolation would be lost by aphid activity after transplanting. A few fields were set with plants from local plant beds except for 12 to 15 rows in the center of the field which were set with plants from the same seed source but grown in isolated areas. These plants were all of the same age and were transplanted (at midseason) and grown under identical conditions. In the fall, 70 per cent of the local-grown plants were infected with mosaic, while less than 1 per cent of the plants from isolated plant beds were diseased. At the time of the spring survey the plants from isolated plant beds had only 4 per cent and 1 per cent mosaic in 2 such fields and were markedly superior in growth and vigor. The yield of seed harvested from the plants from the isolated plant bed in one of the fields was 220 per cent that harvested from a comparable sample from plants grown locally.

The average yield of seed in 1944-45 on 582 acres grown from plants from isolated plant beds was 628 pounds per acre. In the same season the average yield from 485 acres grown from plants from local plant beds was 359 pounds per acre. This constitutes a difference in average yields of 269 pounds per acre.

The 1945-46 Crop.—All of the plants for the 1945-46 crop were grown in well-isolated areas except for 6 small plant beds which provided plants for 100 acres. Four of these small beds were fairly well isolated while 2 were close to heavily infected fields. When this crop was surveyed in October, 1945, cabbage mosaic had been reduced to an almost insignificant status. The average percentage infection of the entire acreage was 2.4 per cent, and for the acreage planted from the local plant beds it was 10.1 per

cent. Not a single field planted from the isolated plant beds had as much as 10 per cent diseased plants; 36 per cent of the fields were free of infection and the average infection in this acreage was 0.9 per cent. Results obtained the previous season indicated that the percentage of infection would remain low until harvest, at least late enough in the second season to avoid serious yield reductions.

DISCUSSION AND SUMMARY

The production of seed of biennial vegetable crops usually entails a system of culture that allows no crop-free period during which disease inoculum and insect pests can be destroyed or reduced. Under such conditions strict sanitation is necessary if a build-up of virus inoculum is to be avoided. Low standards of sanitation under such a system of culture created a tremendous build-up of virus inoculum in the cabbage seed crops of the Puget Sound area. Over 60 per cent of the plants of the entire cabbage seed acreage for 1943 and 1944 harvests were infected with cabbage mosaic. Severe reductions in seed yields resulted. It is difficult to control the cabbage aphid in this area sufficiently to prevent widespread virus infection. Consequently, it was necessary to avoid infection during the period when viruliferous aphids were extremely migratory. Infection of cabbage seed plants was avoided by isolation of plant beds from diseased seed fields.

In seed fields of the 1944-45 crop planted from isolated plant beds, the amount of mosaic 7 to 8 months after transplanting was only 5 per cent of that found in the crop, as a whole, grown in the same area during the 2 preceding years. This reduction is similar in degree when comparison is made with the 1945-46 fields planted from plant beds grown proximal to diseased seed fields. In the 1945-46 crop over 90 per cent of the plants were grown in well-isolated areas and the average infection of the entire acreage 8 weeks after transplanting was only 2.4 per cent. Thus, by plant bed isolation, cabbage mosaic has been reduced to a relatively insignificant status. It is thought that this reduction can be maintained by reasonable care and caution in locating plant beds. Wild cruciferous and non-cruciferous hosts do not appear, as a general rule, to be responsible for more than scattered infections in cabbage fields.

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YIELD REDUCTIONS BY LOOSE SMUT OF WHEAT¹

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The persistence of high levels of infection with loose smut, *Ustilago tritici* (Pers.) Rostr., in the soft red winter wheats of the middle western states during the past decade has frequently raised the question of the relation of the degree of infection to yield losses. This paper reports the results of a study of this question during 1941 and 1942. A study of the effects of loose smut on yield of barley has been published by Semeniuk and Ross (2) and the effects of simulated loose smut losses in wheat by Brown (1).

METHODS AND MATERIALS

Two highly susceptible varieties, Purdue No. 1 and Wabash, were studied in 1941 and 1942. Two lots of seed of each variety from lightly and severely infected fields were obtained from the 1940 crop on the Purdue Soils and Crops farm at Lafayette, Indiana, for use in the 1941 study. The heavily infected seed of Purdue No. 1, studied in 1942, was obtained from a severely infected commercial field at Thorntown, Indiana, and the two lots of Wabash seed and the lightly infected Purdue No. 1 from the Purdue Soils and Crops farm. There was no evidence in the experimental plots of any significant amount of seed-transmitted disease other than loose smut.

In the 1941 studies, all seed was sown at the rate of 6 pecks per acre. In 1942, all Wabash seed and the lightly infected Purdue No. 1 was sown at the 6-peck rate. However, because of low percentage germination, the heavily infected Purdue No. 1 was sown at 9 pecks per acre. All seed was treated with one-half ounce per bushel of New Improved Coresan (ethyl mercury phosphate). The number of heads of each variety (Table 1) in the severely smut-infected plots nearly equalled or exceeded those in the lightly infected plots.

The tests in both 1941 and 1942 were sown in randomized blocks with 10 replications. Each plot consisted of three 18-foot rows from which 16 feet of the center row was harvested for yield determinations. Counts of the numbers of heads produced and numbers of heads smutted were made on 3 feet of one end of a single row of each variety in each replication, after the row ends had been trimmed. The significance of differences was determined by the analysis of variance.

The theoretical yield of each variety on a smut-free basis was derived from the yield of the lightly infected entry corrected for an assumed yield reduction equal to the small percentage of loose smut infection. The yield reduction in a given variety attributable to the severe loose smut infection was calculated as the difference between the yield of these entries and the

¹ Cooperative investigations by the United States Department of Agriculture and the Purdue University Agricultural Experiment Station. (Journal Paper No. 244 of the Purdue University Agricultural Experiment Station.)

TABLE 1.—*Yields of 2 varieties of wheat, lightly or severely infected with loose smut, in 1941 and 1942*

Wheat variety and data obtained	1941		1942 ^d	
	Seed lightly infected	Seed severely infected	Seed lightly infected	Seed severely infected
Purdue No. 1				
Seeding rate per acre (pecks)	6	6	6	9
Head counts				
Numbers	119.8	120.0	75.2	89.5
Percentage smutted	1.3	7.3	4.5	35.9
Actual acre yield ^b (bu.)	41.8	39.2	15.6	10.1
Yield reduction ^c (per cent)	...	7.4	...	38.2
Wabash				
Seeding rate per acre (pecks)	6	6	6	6
Head counts				
Numbers	115.7	107.4	78.7	88.1
Percentage smutted	1.6	13.2	1.9	8.4
Actual acre yield ^b (bu.)	38.3	33.7	17.8	16.7
Yield reduction ^c (per cent)	...	13.4	...	7.9

^a Means of 10 replications, each taken for 3 feet of drill row. Significant differences at the 5 per cent level between total numbers for different treatments = 10.7 heads in 1941 and 10.5 heads in 1942.

^b Means of 10 replications. Significant yield differences between treatments at the 5 per cent level = 2.6 bushels in 1941 and 2.8 bushels in 1942.

^c Percentage of the theoretical yield on a smut-free basis.

^d Severe infestation with Hessian fly accounts for low yields.

theoretical yield on the smut-free basis of the variety concerned. These differences (Yield reductions in table 1) were expressed as the percentages of the theoretical yields.

PRESENTATION OF DATA

In the 1941 tests, the numbers of heads per unit area of plot of the lightly and severely infected entries of each variety were closely comparable, the difference being considerably less than that required for significance. The percentage yield reductions attributable to the severe loose smut infection in both Purdue No. 1 and Wabash closely approximated the percentage of heads destroyed by loose smut. These comparisons involved a 7.4 per cent yield reduction in Purdue No. 1 associated with 7.3 per cent loose smut, and a 13.4 per cent yield reduction in Wabash associated with 13.2 per cent loose smut. The data are presented in table 1. The yield losses were significant at the 5 per cent level.

The results of the 1942 tests corresponded rather closely with those of the preceding year for the variety Wabash (Table 1). A much more severely infected seed lot of Purdue No. 1 was used in 1942. The smut infection in plots from this seed averaged 35.9 per cent and was attended by a yield reduction of 38.2 per cent. This seed, sown at a 9-peck rate, gave numbers of heads per unit area significantly greater than those from the lightly infected seed sown at the 6-peck rate.

DISCUSSION AND CONCLUSIONS

The yield losses of grain, attributable to loose smut in the two wheat varieties studied, closely approximated the percentage of heads infected with loose smut in both 1941 and 1942.

The possibility of confusion in the interpretation of these results because of the possible occurrence of other seed-borne diseases in certain seed lots is not believed to be a significant consideration in this study. In the 1941 study all lots of seeds used were produced on the same farm. No significant infection with any seed-borne disease other than loose smut was to be observed in the plots in either year. Low germination in one seed lot, compensated for by increased seeding rate, was associated with a high proportion of thresher-injured and split kernels.

These results are in agreement with the conclusion of Brown (1) who deduced from experiments on head removal that reductions in grain yield of wheat, resulting from loose smut, would approximate the percentage of loose smut infection. The results are also similar to those of Semeniuk and Ross (2) who, working in Canada and Minnesota, found the percentage yield losses in spring barley to be approximately as great as the percentage of loose smut infection.

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GLOEOSPORIUM VENETUM AND G. NECATOR: TWO DISTINCT SPECIES ON RUBUS¹

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(Accepted for publication September 20, 1946)

An unfortunate assumption that *Gloeosporium necator* Ell. & Ev. was merely another name for *G. venetum* Speg. has resulted in a long-standing confusion in pathological literature relative to anthracnose of brambles (*Rubus*). The present discussion attempts to elucidate the several identities of the two species concerned in the light of the original applications of these names and of subsequent usage.

GLOEOSPORIUM VENETUM SPEG.

The original diagnosis of *Gloeosporium venetum* Speg., published in 1879 (19, p. 477) is as follows:

"*Gloeosporium* (Eugloesp.) *Venetum* Speg. Maculae marginales, amorphae, maximae, vel centrales rotundae parvulae, mellae vel ochraceae linea fusco-purpurascente cinctae. Acervuli minuti, prominuli, gregarii vel solitarii, atri. Conidia ellipto-cylindracea, 7-8 - 2-2½, granulosa et gutturalata, hyalina.

"Hab. Ad folia viva vel languida, Rubi Chamaemori a Belluno & a Conegliano, Aut. 1877-78."

The material of this fungus available to us consists of a fragment obtained by Shear² from the specimen collected by Professor Spegazzini at Belluno, Italy, in October, 1878, and designated as No. 13 of his *Supplemento ad Decades Mycologicae italicae* preserved in the DeNotaris Herbarium at Rome.³ We may consider the fragment as part of the type; it is but a single leaflet (Fig. 1, A and B) bearing fructifications and having the purple-bordered discoloration mentioned in the description.

On the basis of the leaflet at hand we have ascertained that the host of *Gloeosporium venetum* is definitely not *Rubus chamaemorus* L., cloudberry. Instead, it probably is one of the forms of *R. fruticosus* L., the common European species of blackberry.⁴ It seems plausible to assume that Spegaz-

¹ An abstract of this paper for presentation has been published (13).

² In 1912 Dr. Shear, as pathologist in charge of diseases of small fruits in the Bureau of Plant Industry, U. S. Department of Agriculture, visited European mycological herbaria and was given fragments of typical material of fungi on these hosts, including that of *Gloeosporium venetum*.

³ Lindau and Sydow (14, p. 557-559) and Trotter (21, p. 249-257) list *Decades Mycologicae italicae*, issued in 1879, among the publications of Spegazzini; however, we have found no reference to the supplement, nor do we know whether it was distributed to all the original subscribers. It is not with the set in the Division of Mycology and Disease Survey, which is a comparatively recent acquisition. The supplement is also absent from Spegazzini's own set of his *Decades Mycologicae italicae* in the Spegazzini Herbarium in the Instituto Botanico "Spegazzini," Museo, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina. We are indebted to Drs. L. R. Parodi and J. C. Länquist for this information supplied in 1943, together with the fact that there is entirely lacking in Spegazzini's herbarium any reference to the supplement, any specimens from it, or any authentic material of his *Gloeosporium venetum*.

⁴ For this identification we are indebted to Dr. S. F. Blake, U. S. Dept. of Agriculture.

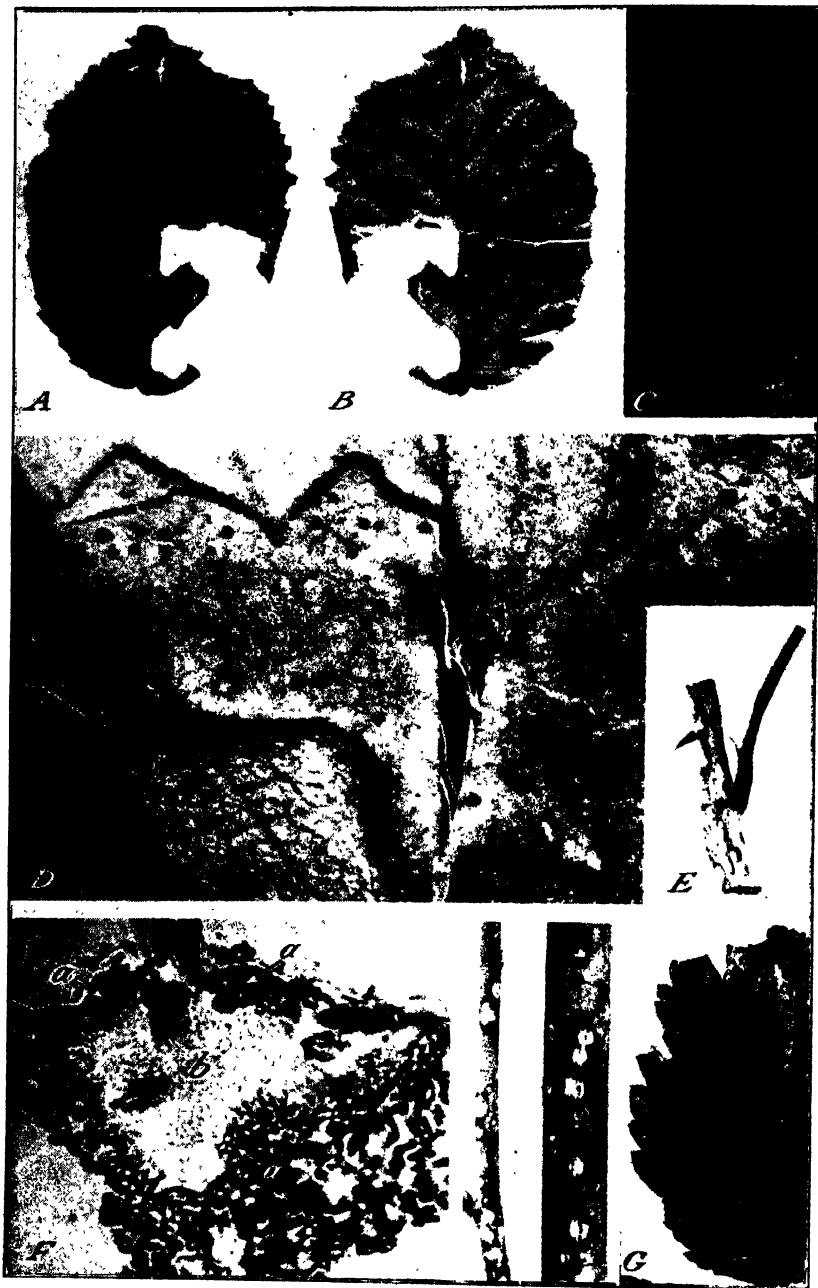


FIG. 1. A-D. *Gloeosporium venetum* Speg. on leaflet of *Rubus*, presumably *R. fruticosus*, Belluno, Italy, October, 1878, C. Spegazzini (Spegazzini, C. Supp. ad Dec. Mycol. Ital. No. 13). A. Upper surface of leaflet showing purple-bordered brown marginal discoloration on which the fungus is fruiting. $\times 1$. B. Lower surface of same leaflet. $\times 1$. C and D. Discolored apical region of the leaflet, showing numerous pycnidia. $\times 12$. E. *Gloeosporium necator* Ell. and Ev. on *Rubus occidentalis*, Anna, Ill., June, 1884, F. S. Earle. Fragment of specimen in Ellis Collection New York Botanical Garden. $\times 3$. F. *G. venetum*, section of pycnidium from C; a, pycnidial wall; b, conidia in pycnidial cavity. $\times 350$. G. Anthracnose on stems and leaflet of wild black raspberry from a woodland, Bluemounds, Dane Co., Wis., July 26, 1938, H. C. Greene and A. E. Jenkins. $\times 1$. Photographs by M. L. F. Foubert (A-D, and G) Lillian Guernsey (E) and Marguerite Wilcox (F).

zini's collection of *G. venetum* at Conegliano was also on *R. fruticosus*. At that place, where the youthful mycologist was living at the time (cf. 21), he collected three other fungi on that particular host. These were reported upon by his friend and teacher Prof. P. A. Saccardo (cf. 15, p. 386, p. 420. and p. 423).

Spegazzini's preparations of his *Gloeosporium venetum* evidently did not reveal to him that the more or less flattened, dark fructification visible on the upper leaf surface is actually a thin-walled pycnidium. This dark body is astomous, more or less flattened, reaching $140-185 \times 48-112 \mu$ in dimensions (Fig. 1, C, D, and F).⁵ Slender unbranched hyaline sporophores, reaching 12μ in length, are produced from practically the entire inner surface of the relatively thin pycnidial wall. Conidia in the preparations measure $4.8-5.8 \times 1.6-2 \mu$. These somewhat smaller dimensions than in the original description could be accounted for by the shrinkage of the material in fixing.

Clearly, to avoid any further confusion, Spegazzini's pycnidial fungus on what is presumably *R. fruticosus* from the Italian Adriatic region should no longer be referred to *Gloeosporium*. Insofar as we know this is the only correct record of *G. venetum* on *Rubus*,⁶ moreover, we have found no described sphaeropsidaceous fungus that seems definitely to apply to it. *Phyllosticta fuscozonata* Thuem. (2, p. 14) on leaves of European red raspberry (*Rubus idaeus* L.), also from the Adriatic region, has certain features in common with Spegazzini's *G. venetum*, such as epiphyllous, lenticular pycnidia and hyaline spores of nearly the same size. On the other hand, marginal discolorations are not described for the *Phyllosticta* leaf spot, nor is the multizonate characteristic of this leaf spot mentioned for that of the *Gloeosporium*. Unfortunately, we have no authentic material of *P. fuscozonata* for comparison with *G. venetum*. The original description of *Phyllosticta fuscozonata* is:

"*Phyllosticta fuscozonata* Thüm. nov. spec.

P[hylosticta]. peritheciis epiphyllis, sparsis, mediis, fuscis, lenticularibus in macula magna, irregularis vel subsinuosa, sordide fusca, griseo-fusca multizonata, ferrugineo indistincte cineta; sporis cylindrico-oblongis, utrinque rotundatis, rectis, plerumque biguttulatis, hyalinis, 7-9 mm. long., 3.5-4 mm. crass.

Ad *Rubi Idaei* Lin. folia viva. Aest.—Plezzo (n. ° 778)."

Under these circumstances, we are suggesting that *Gloeosporium venetum* Speg. may be regarded tentatively as a synonym of *Phyllosticta fuscozonata* Thuem.⁷

⁵ Acknowledgment is here made to Miss Marguerite Wilcox, of the Division of Fruit and Vegetable Crops and Diseases, U. S. Department of Agriculture, for preparing the microscopic mounts employed in this study. These were embedded in paraffin and stained with triple stain.

⁶ Briosi (3, p. 278) reported *Gloeosporium venetum* on wild rose (*Rosa*) growing at Fuipiano, Bergamo, Italy. It would not be expected that the fungus in question on rose was not pycnidial. If the specimen is still in existence it could be examined to determine whether it is not *Sphaeloma rosarum* (Pass.) Jenkins (9, p. 330).

⁷ We should be glad to hear from any one who has an authentic specimen of this fungus. We have ascertained that there is none in the Mycological Collections of the Bureau of Plant Industry, the Farlow Herbarium, Harvard University, or the New York Botanical Garden.

GLOEOSPORIUM NECATOR ELLIS & EV.

In 1888, Prof. F. L. Scribner (16) reported on "Anthracnose of the raspberry and blackberry" then "wide-spread and destructive" in the United States. He noted that Prof. T. J. Burrill (5) of Illinois, had previously published an account of the disease. Scribner continued: "Professor Burrill did not name the fungus, but merely referred to it as the 'Raspberry Cane Rust,' the popular name by which it is known, stating, however, that the parasite probably belonged to the same group as the fungus that caused the disease of grapes known as Anthracnose. . . . In November 1887, Messrs. Ellis and Everhart described the fungus under consideration and, believing it to be a new species, named it *Gloeosporium necator*."

Ellis and Everhart's (6, p. 129) description is as follows:

"*Gloeosporium necator* E. and E.—On living canes of black and red raspberry. Sent from Evanston, Ill., by Chas. Wheeler, August, 1881, and from Cobden, Ill., by F. S. Earle, June, 1884; also received from Columbus, Mo., June 1887, from B. T. Galloway. Spots caulicolous, pale, with a slightly raised dark border, 2–3 millim. in diameter, orbicular or elliptical; spores oblong-elliptical, 5–7 × 3, oozing out in an amber-colored mass through a single opening in the center of each spot. Reported as being very injurious. *G. venetum* Sacc. (sic) [Speg.] has spores of about the same size but is a follicolous species. The Illinois specimens were reported as *Phyllosticta necator*, but the fungus is evidently a *Gloeosporium*."

Unfortunately Scribner (16) in a laudable attempt to avoid duplication of names accepted Spegazzini's earlier name rather than that of Ellis and Everhart. He explained that he had "no specimens for comparison, but the description given by Spegazzini of *Gloeosporium venetum* published in 1877 (sic), applies perfectly to the American fungus described by Ellis and Everhart in everything except that it is said to occur on leaves." He continued:

"In the description of *G. necator* reference is only made to its occurrence on the canes; the fact of its being very common on the leaves was apparently unknown to the authors of the species. Recent observations have led to the discovery that no part of the plant above ground is free from the attacks of the parasite. It is occasionally seen attacking the fruit, and the petioles and veins of the leaves are often greatly disfigured by it."

Scribner's adoption of *Gloeosporium venetum* instead of *G. necator* resulted in the almost complete displacement of *G. necator*, designating a destructive pathogen on *Rubus* from America, by an unknown Italian fungus also on *Rubus*, viz. *G. venetum* Speg. An exception appears in Thaxter's (20) report of "Anthracnose of the raspberry" in Connecticut, in 1889, in which he cited the pathogen as *Gloeosporium necator* Ell. & Ev. "Numerous round or elongate white patches" are produced "on the 'canes,'" he stated, "and small yellowish spots on the leaves." These same symptoms are illustrated in figure 1, G. The small leaf spot is, of course,

entirely distinct from the continuous marginal discoloration produced by the true *G. venetum*.

Ellis evidently made no change in his description when he found that the fungus was "*a Gloeosporium*." In the "Ellis Collection" in the New York Botanical Garden the specimens, except for the Missouri material which is lacking, still appear under the unpublished name *Phyllosticta necator*. A fragment of the specimen "on living black raspberry canes" collected at Anna (near Cobden), Ill., in June, 1884, by Earle has been made available to us for examination through the courtesy of Dr. F. J. Seaver. The numerous small cankers present on the specimen are in keeping with the technical description of them (Fig. 1, E). On their surfaces is the thick exposed stroma on which the "oblong-elliptical conidia" are borne as illustrated by Burkholder (4, fig. 16) for the conidial stage of *Elsinoë veneta*. This conidial stage is typical of the form genus *Sphaceloma* de Bary (1, also cf. 12) where Burrill indicated it should be placed. His difficulty was that he found the conidial stage from bramble so similar to *S. ampelinum* de Bary (1), type of the genus *Sphaceloma* and pathogen of grape anthracnose, that he could not satisfactorily distinguish the two. Scribner (17, p. 133) later referred to the "striking similarity" of these two fungi and published comparable drawings of acervuli (17, figs. 1511 and 1578, reproduced in 10, figs. 1, A and B). Our cultural comparison of these fungi made independently revealed their "very close resemblance in pure cultures" (Shear, 18) and the fact that they were separable when grown under parallel conditions (Jenkins, 7, 11). In cross inoculation experiments neither species affected the host of the other (11).

We are here transferring *Gloeosporium necator* to the genus *Sphaceloma* as *S. necator* (Ell. & Ev.) n. comb. The name *G. venetum* as used by American authors, of course, refers to *G. venetum* Speg.; the true *G. venetum* Speg. on *Rubus* from Italy is entirely inapplicable. The new combination naturally has no effect on the name ordinarily applied to this organism, i.e., its perfect stage name *Elsinoë veneta* (Burkh.) Jenkins—Syn. *Plectodisceella veneta* Burkh. (8, p. 696).

In the absence of a specified type specimen of this fungus we suggest that Earle's specimen on black raspberry (*Rubus occidentalis* L.) be so designated. We have deposited the fragment received from Dr. Seaver in the Mycological Collections of the Bureau of Plant Industry and have indicated that it is "part of the type." The packet containing the specimen in the Ellis Collection is actually made of Professor Earle's letter transmitting the specimen to Ellis. The text of the letter written from Cobden, Ill., on June 17, 1917, reads:

"I enclose you a few young stems of black cap raspberries showing as I think the first stages of the so-called "cane rust." On a very hasty examination I find great numbers of minute simple spores. What is it? I have not been able to find any distinctive fungus in connection with these spots before."

Two days later Earle wrote Ellis again transmitting a specimen of the fungus on "Reliance" red raspberry (*Rubus idaeus*). The Wheeler specimen in the Ellis Collection bears the following label: "*Phyllosticta necator*, Evanston, Ill.?, leg. Charles Wheeler, Aug. 1881. Com. E. Williams, Montclair, N. J." These two specimens could be designated as paratype. We have no knowledge of what became of the cited specimen obtained by Galloway in Missouri. It is not available either in our Mycological Collections or in the Farlow Herbarium and as stated above has not been found in the New York Botanical Garden.

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THE EFFECT OF LIGHT AND TEMPERATURE ON CONIDIUM PRODUCTION BY *HELMINTHOSPORIUM GRAMINEUM* IN CULTURE

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INTRODUCTION

This paper presents the results of a series of trials testing the effect of light and temperature upon conidiophore and conidium production on agar media by the fungus *Helminthosporium gramineum* Rab. the causal agent of the barley stripe disease. As far as the writers are aware the only report of conidium production on agar media was that of Paxton (8) who reported conidium production on corn-meal agar from single-ascospore cultures of what he considered to be the asceigerous stage on barley straw. Negative reports on attempts to induce sporulation by this fungus in culture have been given by Dickson (3), Isenbeck (6), Ravn (9), Vogt (11) and others. Johnson (7) reported on the effects of temperature, light, moisture, aeration, hydrogen-ion concentration, various types of media, plant tissue extracts, yeast extracts, variations in nutrients, and a number of other factors upon sporulation, all his numerous trials giving negative results.

SPORULATION ON ARTIFICIAL MEDIA

Many investigators have reported that this fungus failed to sporulate on artificial media under a variety of environmental and nutritional conditions. The success obtained by Snyder and Hansen (10) in inducing sporulation in *Fusarium* species by exposing cultures to diurnal variation in light suggested the use of such a method with the fungus under consideration. The influence of light upon growth and sporulation of certain fungi has been reported by Coons (2) and Harter (5) who reviewed the early literature on the subject.

In April, 1946, twenty single-spore isolations were made on potato-dextrose agar from diseased leaves from each of six lots of barley plants, each lot having been inoculated² during blossom in 1945 by spores from a different source of *Helminthosporium gramineum*. Ten of each of the six lots of 20 isolates growing in test tube slant cultures on potato-dextrose agar were placed in laboratory light and temperature and the other 10 of each lot were placed outdoors exposed to diurnal changes in environment with the maximum possible light without being in direct sunlight. Growth was obtained from 57 of the 60 tubes held indoors and 55 of those held outdoors. After approximately one month all tubes were examined microscopically. No

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² These inoculations were made by Mr. C. A. Suneson, Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Davis, California.

/ conidia or conidiophores were found in any of the 57 cultures held in the laboratory, while 47 out of 55 of those held outdoors had normal conidia and conidiophores. There was, however, considerable variation between isolates with regard to the number of spores produced. Many of the conidia had germinated to produce secondary conidia and mycelium, which indicated that the primary spores had been produced early in the experiment. Ten

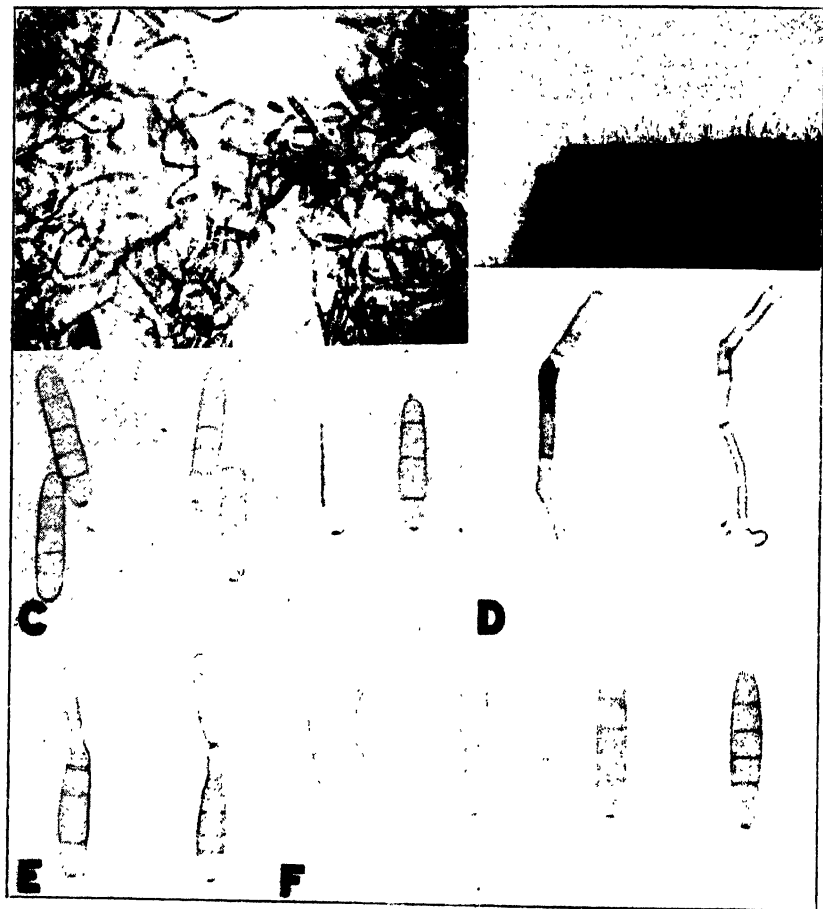


FIG. 1. Conidium production by *Helminthosporium gramineum* Rab. in outdoor environment on agar media and on diseased barley leaves. (A) Conidia and conidiophores produced on potato-dextrose agar containing sterile diseased leaf pieces ($\times 60$). (B) Section of agar block showing conidium production ($\times 16$). (C) Conidia produced on potato-dextrose agar ($\times 255$). (D) Conidiophores produced on potato-dextrose agar ($\times 255$). (E) Conidia showing formation of secondary conidia on potato dextrose agar ($\times 255$). (F) Conidia from naturally infected barley leaves ($\times 255$).

single-spore isolations were made from an individual colony of each of the six lots of single-spore colonies, and conidia developed readily in all 60 of these under the outdoor environment within a week's time.

When those grown indoors were six weeks old the heavy aerial mycelial growth was scraped away from the surface of the agar cultures. A small

piece of the agar, 5 to 8 mm. across, containing imbedded mycelium was then transferred from each single-spore culture to potato-dextrose agar in Petri dishes and these were placed in the outdoor environment. The dishes were sealed against dust with masking tape, a method of sealing used in all subsequent tests. After three days, examination showed that 48 of the 57 pieces of inoculum were producing conidia. At the end of seven days all 57 transfers were producing abundant conidia and conidiophores of a type typical of this species (Fig. 1; B, C, D, E.).

Three isolates originally obtained by single-spore isolation from stripe diseased barley in 1944, carried by mass transfers during the two intervening years, and known to be pathogenic when tested by the method described by Army and Shands (1), were transferred and placed in the outdoor environment. To test their relative value for conidium production, different media were used, namely, potato-dextrose agar, corn-meal agar, water agar, potato-dextrose agar and water agar to which were added several diseased leaf pieces prior to autoclaving, and potato-dextrose agar and water agar plus water extracts from diseased leaves.

Transfers of each isolate were made to test-tube slants of these media and allowed to grow outdoors for approximately three weeks before they were examined for conidia. Conidiophores and conidia were found to have developed in large numbers on those media with the highest nutritional value, namely, normal potato-dextrose agar and on this agar to which leaf pieces or leaf extracts had been added. On the less nutritive media to which leaf pieces were added the colonies developed conidia only when they grew in contact with or near such leaf pieces. The best sporulation was obtained on potato-dextrose agar to which was added the diseased-leaf pieces (Fig. 1. A). Fifty single-spore isolations were made on potato-dextrose agar from one isolate and all appeared to be similar in growth type and pigmentation. Sporulation was obtained on all of these after 1 to 2 weeks outdoors. It was noted that occasional branched conidiophores developed on the aerial mycelium of these cultures. Since little advantage was obtained from using other media, potato-dextrose agar was used in all subsequent trials.

THE INFLUENCE OF LIGHT AND TEMPERATURE UPON SPORULATION

The effect of variations in light and temperature on several isolates known to sporulate well under outdoor environment was studied in May and June at Davis, California.³ During this period the length of daylight was 14 to 15 hours and the average maximum and minimum outdoor temperatures were 26.8° and 8.2° C., respectively. The average maximum and minimum indoor temperatures were 25.7° and 18.0° C., respectively. Duplicate mass transfers of several nonsporulating cultures of these isolates were made to potato-dextrose agar in Petri dishes. A parallel series was carried out with pieces of nonsterilized, dried, diseased leaves placed on potato-dextrose

³ Similar, independently conducted, unpublished studies on other fungi have recently been made by H. N. Hansen and W. C. Snyder, University of California, Berkeley, California.

agar in Petri dishes. Conidiophore and conidium production were recorded separately, because under certain conditions conidiophores were produced but few conidia developed. The results of the trial with diseased leaves were recorded under two headings according to whether sporulation occurred on the diseased leaf tissue or on the new mycelium which grew from the leaf pieces into the agar. These results are in table 1.

TABLE 1.—*Environmental factors governing sporulation of Helminthosporium gramineum*

Environment	Nonsporulating cultures transferred to potato-dextrose agar		Leaf pieces from diseased barley on potato-dextrose agar			
	Conidio-phore production ^b	Conidium production ^b	Directly on diseased leaves	On new mycelium from leaves	Conidio-phore production	Conidium production
Outdoors ^a	+++	+++	+++	+++	+++	+++
Outdoors, darkened	0	0	+++	+++	0	0
Outdoors, daytime; Dark, 25° C., night	+++ ^c	+	+++ ^c	+	+++ ^c	+
Outdoors, daytime; Dark, 12° C., night	+++	+++	+++	+++	+++	+++
Dark, 25° C., 12 hr.;						
Dark, 12° C., 12 hr.	0	0	+++	+++	0	0
Inside light and temperature	0	0	+++ ^c	+	0	0
Artificial light, 13° C.	++ ^c	+	+++	+++	++	+
Artificial light, 13° C., 15 hr.; Dark, 13° C., 9 hr.	+++	+	+++	+++	++	+
Dark, 8° C.	0	0	+++	+++	0	0
Do, 13° C.	0	0	+++	+++	0	0
Do, 18° C.	0	0	+++	+++	0	0
Do, 25° C.	0	0	+++ ^c	+	0	0
Do, 30° C.	0	0	+++ ^c	0	0	0
Do, 35° C.	0	0	0	0	0	0

^a Subject to diurnal changes in light and temperature at Davis, California, during May, 1946, but not in direct sunlight.

^b Number of pluses indicates relative amount of conidiophore and conidium production.

^c Extremely long conidiophores, very few bearing conidia.

On the cultures held outdoors, sporulation was abundant within 48 hours on agar, within 24 hours on leaf pieces, and within 48 hours on the new mycelium from the leaf pieces to within 5 or 6 mm. of the advancing margin. In the parallel series of cultures placed outdoors but darkened by black paper wrapping, conidiophores and conidia were not produced on the agar transfers nor on the mycelial growth from the leaves, but normal and abundant sporulation occurred directly from the mycelium in the leaf pieces. All of the cultures including the leaf pieces placed outdoors in day temperatures and light and transferred at night to a dark incubator at 25° C. produced an abundance of conidiophores $1\frac{1}{2}$ to 2 times their normal length, but only 5 to 10 per cent bore conidia. However, a similar series placed

at night in a dark incubator at 12° C. produced normal and abundant conidiophores and conidia identical with those left outdoors at night.

A series held in incubators in complete darkness at 25° C. for 12 hours and then changed to 12° C. for the remaining 12 hours each day failed to produce conidiophores and conidia on the agar transfers or on the mycelium growing from the leaf pieces; however, normal sporulation occurred directly on the leaves. These results were identical with those in the series held outdoors for 24 hours each day but continuously in the dark. In a series held under laboratory light and temperatures the only sporulation obtained was directly upon the leaf pieces. Here again, as with those placed outdoors during the day and at 25° C. at night, the conidiophores were excessively long and few bore conidia. These long conidiophores with few conidia were probably the result of the higher night temperatures in the laboratory. In continuous artificial light at a constant temperature of 13° C. a moderate number of conidiophores developed on the agar cultures and mycelium from the leaves, but they were slightly longer than normal and only 10 to 15 per cent of them bore conidia. Abundant sporulation occurred directly on the diseased leaf pieces. A similar result, but with conidiophores more nearly normal in length, was obtained with the series at 13° C. with 15 hours of artificial light and 9 hours of darkness each day.

The remainder of the data presented in table 1 comprises trials in complete darkness at six constant temperatures ranging from 8° to 35° C. No conidiophores developed at any of the temperatures on the agar cultures or on the mycelial growth from the diseased leaf pieces. Abundant conidiophore and conidium development occurred on the leaves at 8°, 13°, and 18° C. The conidiophores were very short at 8°, appearing to be even shorter than the conidia. Conidiophores became progressively longer and conidia progressively shorter as the temperature increased. Secondary conidia developed abundantly at 13° and 18° C. At 25° C. conidiophore production was as abundant as that at the lower temperatures but the conidiophores ranged up to once again as long as those produced at 18° C. and only 10 to 15 per cent terminated in conidia. At 25° and 30° C. mycelial growth from the leaves was very profuse. No conidia developed at 30° C., but conidiophores were abundant and were 3 to 4 times the length of those produced at 18° C. At 35° C. there was no mycelial growth from the leaves. Abnormal, light-colored, narrow hyphae somewhat resembling conidiophores grew from the leaf but no conidia were produced.

Measurements were made of 50 conidia produced by a culture on agar medium and of conidia produced at 18° C. on diseased leaves. An attempt was made to measure only primary conidia by making the spore mounts at a time during conidium production when the primary conidia were mature and readily separated from the conidiophores and when only a few scattered secondary conidia had developed. Those from the culture had an average septa number of 4.2 and average size of 70.1 by 16.4 μ with a range in length from 48.6 to 97.6 μ . Conidia from the leaves averaged 4.4 septa and 79.3

by $14.9\ \mu$ with a range in length from 62.4 to $96.8\ \mu$ (Fig. 1, F). Thus, the two lots were very similar and compared favorably with measurements given by Drechsler (4) for this fungus.

As noted above, differences in length were observed in conidia produced from diseased leaves at different temperatures. The longer spores were produced at the lowest temperature. One-hundred conidia each were measured from lots produced at 8° and 18° C., respectively. Those produced at 8° C. had an average number of septa of 5.7 and measured 108.2 by $18.6\ \mu$ with a range in length from 82.6 to $129.0\ \mu$. Those produced at 18° C. were considerably smaller with an average number of septa of 4.4 and dimension of 79.3 by $14.9\ \mu$ with a range in length from 62.4 to $96.8\ \mu$.

DISCUSSION

With the cultures used the results indicate that exposure to light, preferably daylight, is necessary for conidiophore and conidium development on agar. This was true regardless of type of nutrient within the range of those tested and found favorable for good vegetative growth. The agar cultures behaved identically with the mycelium produced from diseased leaves yet the mycelium within the diseased leaves was capable of producing conidiophores and conidia in the dark within a few hours. This indicated that the mycelium within the leaf as a result of the action of diurnal changes of outdoor environment during its development, had stored within it the necessary potentialities, whether chemical, nutritional, or otherwise, for conidium production. These potentialities were not transmitted to the mycelium growing from these leaf pieces into the agar. There was evidence indicating that a moderately high temperature during the period of light and a lower temperature during the period of darkness were conducive to best sporulation. Under these conditions sporulation on agar cultures was obtained within 48 hours. There was some evidence to indicate that an extended period of light as well as the higher temperatures employed resulted in excessively long conidiophores.

The ability to induce this fungus to sporulate in culture will make it possible to maintain single-spore isolates in a study of physiologic specialization, and to have a supply of conidia at all times for use in inoculation. It also makes it possible to positively and rapidly identify the organism when isolated in culture.

SUMMARY

Abundant, normal sporulation was obtained within 48 hours on agar cultures of the fungus *Helminthosporium gramineum* Rab., the causal agent of the barley stripe disease, when held outdoors to expose them to diurnal changes of environment. Conidium production under these conditions was obtained on single-conidium isolations, on mass mycelial transfers, and on mycelial growth which developed from pieces of diseased leaves placed on agar.

A number of media were employed to test their relative value for sporu-

lation, but there was little evidence to indicate that any were superior to ordinary potato-dextrose agar. Various light and temperature combinations were employed in an attempt to determine the effect of each upon sporulation.

In the absence of light no sporulation was obtained on agar slant cultures or on mycelium growing from diseased leaves, either outdoors or indoors. In the presence of light but under continuous high indoor temperatures, again, no sporulation was obtained.

Light, preferably outdoor daylight, was necessary for the induction of sporulation. A few spores were produced under artificial light.

Relatively high temperatures throughout the growing period as well as extended periods of light resulted in excessively long conidiophores few of which produced conidia. This was true of agar cultures and of pieces of diseased leaves. A temperature drop during one-half of each 24-hour period gave best results. When diseased leaf pieces were placed on potato-dextrose agar and incubated in the dark at various temperatures from 8° to 35° C. to obtain sporulation it was found that conidiophore length increased and conidium length decreased as the temperatures increased.

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PHYTOPATHOLOGICAL NOTES

*The Use of Wetting and Adhesive Agents to Increase the Effectiveness of Conidial Suspensions for Plant Inoculations.*¹—In the course of studies on the rice “blast” disease, considerable difficulty was encountered in establishing infection and in securing a uniform distribution of leaf lesions on rice plants inoculated with aqueous suspensions of the conidia of the causal organism, *Piricularia oryzae* Cav. This was because of the high surface tension of the droplets on the waxy surfaces of the rice leaves. When the rice plants were subjected to continued wetness in the humidity tent, free moisture formed on the leaves in large droplets which, as they rolled off, removed conidia which had not yet had sufficient time to germinate and infect the plant. As a result, the leaf infections were few and generally aggregated either at the tips or at the bases of the leaves. In order to increase the effectiveness of atomizing conidial suspensions onto the plants, a series of experiments was conducted using wetting and adhesive agents, singly and in combination. The criteria used for selecting these agents were the effective reduction of surface tension, the adhesion of the solution to the plant, the non-toxicity of the agent to the conidia, and finally, the relative amount of infection resulting from the use of conidial suspensions containing the agent.

The following wetting and adhesive agents were tested by atomizing 1 per cent solutions on rice leaves: soft soap, Castile soap powder, saponin, sodium oleate, gum arabic, Micro-bentonite, Fuller's earth, calcium caseinate, gelatin, and Casco glue. In addition, single droplets of known volume were placed on rice leaves and the areas covered by the droplets were compared. By this method soft soap, Castile soap powder, and sodium oleate were selected as possible wetting agents; and calcium caseinate, Casco glue, and gelatin were selected for possible use as adhesive agents. The lowest effective concentration was then determined for each of the selected solutions and these, in turn, tested singly and in combination for relative toxicity to *Piricularia oryzae* by germinating the conidia in the respective solutions and studying their reactions. At the same time their relative effectiveness on plants was determined by taking equal volumes of suspensions containing an equal number of conidia and atomizing them onto plants. After the inoculated plants were dry they were subjected to 24 hours of continued wetness in a humidity tent to insure the establishment of infection. Further experiments were made using those agents selected from these tests. The results from the germination studies and the last two plant inoculation trials are given in table 1.

It was possible to increase the number of leaf infections per plant many times over that effected by conidia in a water suspension, and, at the same time, to obtain a more uniform distribution of leaf infections over the entire

¹ Work conducted at Camp Detrick, Frederick, Maryland, from September, 1944, to May, 1945.

TABLE 1.—*The effect of wetting and adhesive agents upon the germination of Piricularia oryzae conidia and the establishment of infection on rice plants**

Wetting and adhesive agent	Germination of conidia (per cent)	Trial A		Trial B	
		Total No. plants	Av. No. infections per plant	Total No. plants	Av. No. infections per plant
Water (Control)	99	45	0.2	38	0.8
0.1 per cent soft soap + 0.1 per cent gelatin	97	33	9.5	38	2.1
0.1 per cent soft soap + 0.25 per cent gelatin	96			38	3.8
0.05 per cent Na oleate + 0.1 per cent gelatin	90	44	5.8	38	5.3
0.05 per cent Na oleate + 0.25 per cent gelatin	98	37	11.1	38	6.5
0.05 per cent Na oleate + 0.25 per cent Ca caseinate	90	42	3.1	38	0.7

* Aetia rice in the 4-5 leaf stage was used.

surface of the leaf. Because of this uniformity in lesion distribution, a considerably more accurate and reliable method of determining numbers of leaf lesions was achieved, and this was used as a scoring procedure in later studies.² A solution containing 0.05 per cent sodium oleate and 0.25 per cent gelatin was the most effective spreader-sticker combination. All of those shown in table 1, however, were superior to the water control. Stock solutions of sodium oleate and gelatin were kept in cold storage so that conidial suspensions could be readily made when necessary. It is believed that these combinations of wetting and adhesive agents may prove useful for the application of conidial suspensions to leaf surfaces similar to those of rice.—AXEL L. ANDERSEN and B. W. HENRY.

*Systemic Infection of Downy Mildew in Soybean and Alfalfa.*¹—In the soybean nursery at Madison, Wisconsin, in 1945 downy mildew (*Peronospora mancharica* (Naum.) Syd.) developed about the middle of June and spread to the entire nursery before the end of the summer. In the more susceptible varieties pods at many of the nodes bore seed encrusted with oospores of the fungus as described by Johnson and Lefebvre.² From such seed planted in the greenhouse infected seedlings have at times been obtained with lesions apparently connected by mycelium in a systemic infection. Young plants with such systemic infection have been collected in the field by Dr. W. B. Allington and others, and in the collection by Allington characteristic mycelium of the fungus has been found in all parts of the plants through the hypocotyl and first trifoliate leaf.

² Andersen, A. L., B. W. Henry, and E. C. Tullis. Factors affecting infectivity, spread, and persistence of *Piricularia oryzae* Cav. Phytopath. In press.

¹ A cooperative investigation of the U. S. Department of Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, and the Wisconsin Agricultural Experiment Station.

² Johnson, H. W., and C. L. Lefebvre. Downy mildew on soybean seeds. U. S. Dept. Agr., Pl. Dis. Rptr. 26: 49-50. 1942.



FIG. 1. Types of downy mildew lesions on soybeans. A. Noninfected leaflet; B. Leaflet of a variety having uniformly small lesions; C. Leaflet of a variety having large and often coalescent lesions.

Varieties of soybeans differed in the kind and the abundance of lesions that developed in the field during the summer; and the same difference was found when these varieties were inoculated in the greenhouse early in the following spring. In some varieties visible lesions were absent or rare in the

TABLE 1.—*Classification of soybean varieties based upon size or number of downy mildew lesions, and the percentage of encrusted seed produced in a nursery in 1945 at Madison, Wisconsin*

Variety	Character of lesion	Encrusted seeds Per cent	Variety ^b	Character of lesion
Rienland	Large	23.8	Goldsoy	Large
Illini	do	18.8		
Earlyana	Small	2.3	Flambeau	Small
Habaro	do	0.5	Kabott	do
Ottawa Mandarin	do	0.3	Pridesoy	do
Mandarin No. 7	do	0.8		
Dunfield	Rare or absent	1.0	Montreal	Rare or absent
Manchu 3	do	1.8	Manchu	
Manchu 606	do	0.5		
Marchukota	do	0.3		
Ontario	do	0.3		
Lincoln	Large or small ^c	7.0		
Mingo	do ^c	3.8		

^a Based upon 400 seeds drawn from each of 4 replicates in the nursery.

^b The five varieties listed were tested in the greenhouse only.

^c Plants within the variety were of two kinds, one kind with large lesions, the other with small lesions.

field, and none were found in the greenhouse; in others, lesions on all plants were large (Fig. 1, C) or small (Fig. 1, B) or the variety was a mixture of plants having large or small lesions (Table 1). Varieties and selections having large lesions had outstandingly large percentages of spore-encrusted seed, reaching 41 per cent in one selection. On the other hand varieties in which lesions were rarely found still had occasional spore-encrusted seed. Whether these were borne on plants with rare or no visible lesions or came from seed mixtures remains to be determined.

Mildew infection apparently had no effect on seed yield in this nursery. A nonsignificant correlation coefficient of -0.17 ($N = 67$) between percentage of seeds encrusted with mildew and yield of seed in bushels per acre was obtained from a yield nursery of segregates of (Lincoln \times Richland) Lincoln.

A nursery of transplanted alfalfa was also infested with mildew (*Peronospora trifoliorum* DBy.) in 1945. The nursery contained clones of unselected plants from several varieties and strains, and also closely spaced single plants obtained from selfing selected plants whose susceptibility to mildew was known. Clones and plants differed as in soybeans. Many were not infected at all. Infected plants usually had only infected leaves. A few had systemic infection to some degree, sometimes only in axillary shoots in late fall, but more often in late fall in crown shoots which were pale in color, but which survived the winter, and on which conidia were found April 13, 1946. These conidia are assumed to be the source of heavy infestation in this nursery in the following May.

The percentage of infected plants in varieties ranged from 12 per cent in Hardistan and Ladak to 60 per cent in Hardigan. Among the populations obtained by selfing susceptible plants, one of 90 plants developed in late autumn systemic infection in one or more crown shoots of each individual. The parent was infected in the same manner. Parents susceptible to leaf infection gave populations in which over half the plants were similarly infected, with a small percentage of plants in which systemic infection occurred also. Both systemic and leaf infection has been obtained from inoculation in the greenhouse; a thorough comparison of the reaction of plants in the greenhouse with that in the field has not been made. However, it appears at present that systemic infection in shoots is an important agency in the overwintering of this fungus, and that but a part of the plants that may have leaf infection are capable of this systemic invasion.—FRED R. JONES, Senior Pathologist, U. S. Department of Agriculture, and J. H. TORRIE, Associate Professor of Agronomy, Wisconsin Agricultural Experiment Station.

INDEX FOR VOLUME 36

AUTHOR AND SUBJECT INDEX

New species in **blackface type**

Junior authorship indicated by pages in "()"

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- Abscission, induced by illuminating gas, 505
- Acalypha virginica*, 653
- Acrostalagmus zeosporus* sp. nov., parasitic on nematode, 216
- Actinomyces ipomoea*, cause of soil rot of sweet potato, 869
 - pigmentation and pathogenicity correlated, 411
 - scabies, 388
- Actinomyces*, on citrus, 398
 - and Panama disease of banana, 406, 983
- Adaptation, of *Gibberella zeae* in relation to genetic variation, 396
 - of *Ustilago zeae* to arsenic, 411
- Adelphocoris lineolatus*, 514
- Adelphocoris*, 514
- Adlumia fungosa*, 654
- AFANASIEV, M. M., 394
- Ageratum conyzoides*, 647
- ALBERT, W. B., 703
- Alfalfa, 348, 634
 - downy mildew, 1057
 - effect of temperature on *Rhizoctonia* root canker, 638
 - viability of treated seed, 403
 - virus diseases of, 117, 142, 401, 762
 - water-congestion, 402
- ALLEN, T. C., (504)
- ALLINGTON, WILLIAM B., 319, 386, 394
- Allium ascalonicum*, virus of, 292
 - cepa, 653 (See also Onion)
 - var. *solanium*, 292, 297
 - var. *viviparum*, 292
 - fistulosum*, 294
 - sativum*, virus of, 292
 - virus 1 (See Viruses; onion yellow dwarf)
- Almond, 284
- Alternaria, 679
 - citri*, effect of diphenyl vapor on, 888
 - effect of ultraviolet radiation on spores of, 102
 - radicina*, effect of diphenyl vapor on, 888
 - relation of fertilizers to defoliation of tomato by, 681
 - solan*, on tomato, fungicides for control of, 681
 - effect of diphenyl vapor on, 888
 - tomato, effect of diphenyl vapor on, 888
- Althaea rosea*, 654
- Amaranthus tricolor*, 646
- Ambrosia trifida*, 647
 - Pythium* on, 782
- AMERICAN PHYTOPATHOLOGICAL SOCIETY, annual meeting report, 574
- New England Division, report of annual meeting, 679
- Potomac Division, report of annual meeting, 684
- Anagallis arvensis*, 650
- Anchusa azurea*, 654
 - capensis*, 654
- ANDERSEN, A. L., 1056
- ANDERSON, M. S., (554)
- ANDERSON, P. J., 679
- ANDES, J. O., 394
- Angiospora zeae*, 412
- Ansatospora acerina*, 893
 - bromi*, 895
- Antirrhinum majus*, 650
- Aphanomyces*, on sugar beet, 394
 - cladogamus*, 845
- Aphelenchoides fragariae*, on fern, 892
- Aphids, as vectors of alfalfa virus, 142
- Aphis rumicis*, 514
- Apium graveolens*, 652
 - var. *dulce*, 980
- Apple, 284
 - blotch, control of, 572
 - Brooks' fruit spot, control of, 236
 - geographical distribution of *Elsinoë piri* on, 458
 - leaf structure in relation to spray penetration, 681
 - measles, 395
 - scab, control of, 236, 572
- App-L-Set, hormone dust, 505
- Apricot, leaf injury from fluorine, 469
- Aquilegia caerulea*, 654
- Arachis hypogaea*, 345
- Arasan (See Fungicides)
- Aretium lappa*, 647
- ARK, P. A., 549, 695, (699), 758, 865, 892
- Armillaria mellea*, relation of temperature to pathogenicity of, 302
- ARNOLD, C. H., 24, 30
- Aselepias curassavica*, 653
 - syriaca*, 653
- Asparagus officinalis*, wilt and root rot of, 397
- Aspergillus*, 679
 - effect of diphenyl vapor on, 888
 - on corn seed, control of, 93
 - on cotton seed, 29, 34
- Asplenium nidus*, 758
 - nematode of, 892
- Atropa belladonna*, 688
- Avena* spp. (See Oats)
- B-72 (See Fungicides)
- Bacillus farinetianus*, 696
- pollacii*, 696

- Bacteria, association with wheat roots, 277
 dissociation of, 589, 613
 overwintering, 677
 seed transmission of blight of sugar beet, 549
 soft rot in potato tubers, ring-rot symptoms, 237
 Bacterial blight of cotton, 409
 Bacterial disease of orchids, 695
 Bacterial leaf blight of fern, 758
 Bacterial leaf spot of gardenias, 865
 Bacterial pustule of soybean, 405
 Bacterium medicaginis var. phasecolicola, 277
 krameriani, 696
 BAKER, G. A., (418)
 BAKER, KENNETH F., 281, 380, 493
 Banana, Actinomyces and Panama disease of, 406, 983
 Barbaroa vulgaris, 648
 Bailey, foot rot of, 397
 reaction of varieties to loose smut, 534
 seed microflora of, 399
 seedling infection by *Xanthomonas translucens* var. *cerealis*, 446
 stripe, method of inoculation, 689
 water-congestion and infection, 402
 BARRATT, R. W., 679, (680)
 BARRETT, J. T., (865)
 BARRETT, JAMES W., (397)
 Basicop (See Fungicides)
 Bean, Broad, powdery mildew of, 370
 infection by *Phytophthora aptata*, 552
 Castor, seed treatment, 689
 curly-top virus in, 462
 growth regulating effects of fungicides, 686
 Kidney, infection by *Phytophthora aptata*, 552
 soybean and tobacco viruses on, 321
 Lima, injury by Lygus bug, 493
 mosaic, 170
 overwintering of bacterial pathogens, 677
 Macrophomina phaseoli on, 979
 mosaic virus, associated with bacteria, 589
 new strain, 324
 No. 2, 394
 No. 4, electrophoretic study, 137
 rust, control with sulphur, 689
 String, injury by Lygus bug, 515
 reduction of bud, blossom, and pod drop by hormones, 504
 water-congestion and infection, 402
 Beet, 679, 680 (See also Sugarbeet)
 Begonia semperflorens, 653
 Bellis perennis, 647
 Benzoic acid derivatives, in reduction of abscission, 505
 Benzyl salicylate-cottonseed oil (See Fungicides)
 BERG, ANTHONY, 395.
 BERKELEY, G. H., (73)
 Beta vulgaris, 647
 var. cicla, infected by *Phytophthora aptata*, 552
 Bichloride of mercury (See Fungicides)
 Bidens frondosa, 647
 Biographies,
 CHALMERS, JACKSON KING, 587
 EDGECOMBE, ALBERT EDWARD, 329, 584
 GAINES, EDWARD FRANKLIN, 87
 HARVEY, RODNEY BEECHER, 588
 JOLIVETTE, JAMES PETER, 415, 584
 JONES, LEWIS RALPH, 1, 585
 LEONTIAN, LEON HATCHIG, 241, 586
 WAITE, MERTON BENWAY, 175, 586
 WEIR, JAMES ROBERT, 487
 Bismuth subsalicylate (See Fungicides)
 BLACK, L. M., (157)
 BLISS, DONALD E., 302
 BLODGETT, EARLE C., 675
 Blueberry, virus stunt of, 684
 Bluegrass (See Grasses)
 Blue mold, of sweet cherries, treatment with ultraviolet radiation, 107
 of tobacco, control, 684
 Borax, control of citrus fruit decay, 398
 (See also Fungicides)
 Bordeaux mixture (See Fungicides)
 BORLAUG, N. E., 395, 479
 Boron deficiency of sweet potato, 164
 Botrytis allii, effect of diphenyl vapor on, 888
 cinerea, causing pole-rot of tobacco, 679
 effect of diphenyl vapor on, 888
 effect of ultraviolet radiation on spores of, 102
 BOYLE, L. W. (458)
 Brachycome ibericifolia, 647
 Brassica napobrassica, 648
 oleracea, 408, 648
 pekinensis, 648
 rapa, 340
 BECHER, F. S., (685)
 Breeding (See also Resistance)
 of disease resistant oats, 688
 BRIERLEY, PHILIP, 292, 297
 Bromegrass (See Grasses)
 2-Bromo-3-nitro benzoic acid, 505
 Browallia americana, 650
 speciosa, 650
 BRUNSON, A. M., (412)
 BRYANT, L. R., 329
 BUDRER, EDNA M., (180)
 Bunt, (See Smut; hant)
 C 119 (See Fungicides)
 Cabbage, 679, 680
 control of mosaic, 1035
 improvement of ascorbic acid content, 398
 resistance to mosaic, 408
 seed treatment, 729
 CALDWELL, R. M., (1040)
 Calendula officinalis, 647
 Callistephus chinensis, 647
 Calonyction aculeatum, 648
 Camellia, flower blight of, 380
 Campanula spp., hosts for tobacco viruses, 646
 Cankers, of deciduous trees, 408
 of poplar, 148
 Cannabis sativa, 937
 Cantaloupe, 944

- Capsella bursa-pastoris*, 648
Capsicum annuum, infected by *Phytophthora*
 aptata, 552
 frutescens, 650
Carica papaya, 647
Carnation, 687
Carneccephala fulgida, 118, 401
 triguttata, 634
Carrot, seed treatment, 729
 water-congestion, 402
CARTEK, J. C., 395
Cassia nictitans, *Macrophomina phaseoli*
 on, 979
Castanea crenata, 554
Castor bean, seed treatment, 689
Casuarina stricta, *Armillaria* root rot of,
 302
Cattleya, 695
Celery, resistant to *Cercospora apii*, 980
 water-congestion, 402
Celosia argentea, 646
Centaurea spp., hosts for tobacco viruses,
 647
Centrospora bromi, 895
 Ohlensii, 895
Cephalosporium, effect of diphenyl vapor
 on, 888
Ceratoma trifurcata, 514
Ceratostomella fimbriata, 284
 radicicola, 284
 ulmi, in Massachusetts, 680
 rate of spread of, 689
Cercospora apii, on celery, 980
 oryzae, races of, 395, 950
Cereals, effect of crop rotation on root rot,
 410
Ceresan (See Fungicides)
Cetyl isoquinolinium bromide (See Fungi-
 cides; Isothan)
Chaenomeles japonica, 284
Chaetomium, on cotton seed, 29
Chalara quercina, 397
Chalaropsis thielavioides, causing bud and
 graft union failure in rose, 281
Charliea heterophylla, 647
Cheiranthus allionii, 648
 cheiri, 648
Chelidonium majus, 649
Chemotherapy, against bacterial blight of
 pear and walnut, 717
 Oxyquinolin benzoate, effect on foliage
 wilting of elm, 682
Chenopodium album, 647, 688
 ambrosioides, 647
 glaucum, 647
 rubrum, 647
Cherry, relation of temperature to yellows
 virus, 353
 sour, relation of yellows to yield, 406
 sweet, 284
 tatter-leaf virus of, 73
 ultraviolet radiation to control fungus
 decay, 100
 virus of, 409
Chestnut, nutrition related to blight sus-
 ceptibility, 554
CHILDS, LEROY, (778)
CHILTON, ST. JOHN P., 395, 950
CHITWOOD, B. G., 180, 684
Chloranil, 680
 2-Chloro-5-nitro benzoic acid, 505
 p-Chlorophenoxy acetic acid, 505
Chondriosomal changes in *Hosta japonica*,
 472
CHRISTENSEN, J. J., 396
CHRISTIE, JESSE R., 340
Chrysanthemum, 687
 leucanthemum, 647
Cichorium endivia, 647
Citrivir pertinaciae (See Viruses; stubborn
 disease)
Citrullus vulgaris, 653
Citrus aurantium, *Armillaria* root rot of,
 302
Citrus, control of fruit decay, 398
 necrosis and gummosis of, 398
 sinensis, *Armillaria* root rot of, 302
Cladosporium, 679
 cucumerinum, effect of diphenyl vapor
 on, 888
 herbarium, effect of ultraviolet radiation
 on spores of, 102
 effect of diphenyl vapor on, 888
Clarkia elegans, 654
CLAYTON, E. E., 684
Cleome spinosa, 647
Clover, Ladino, viability of treated seed,
 403
 red, viability of treated seed, 403
 water-congestion, 402
CLULO, GENEVIEVE, (395)
Cobaea scandens, 650
COCHRAN, G. W., 396
COCHRAN, L. C., 396
COHEN, SYLVAN IRVING, 397
Coix lachryma-jobi, inoculation with smut,
 411
Coffee weed, 979
Coleus blumei, 649
Colletotrichum circinans, effect of diphenyl
 vapor on, 888
 gloeosporioides, effect of diphenyl vapor
 on, 888
 gossypii, survival in stored cotton seed,
 24
 lilii, control of, with fungicides, 391
Collinsia bicolor, 650
COMPTON, LEROY, 1040
COOK, HAROLD T., 397
Copper compounds (See Fungicides)
Coreopsis grandiflora, 647
Corn, 979
 diseases of in Guatemala, 412
 dwarfing and witches' broom of, 410
 ear rot caused by *Physalospora zeae*, 201
 inheritance of susceptibility to *Hel-*
 minthosporium carbonum, 412
 relation of pericarp injury to disease,
 403
 resistance to *Helminthosporium turei-*
 cum, 660
 seed treatment of, 93, 729
 water-congestion and infection, 402
Corticium solani, 401
 pathogenic on yellow calla, 699
 vagus, cause of foot rot of milo, 410

- Coryneum asperulum* sp. nov., 75
beijerinckii, effect of diphenyl vapor on, 888
 Cotton, bacterial blight of, 409, 413
 loss of viability of stored seed, 30
 microflora of seed of, 24, 30
 nutrition and wilt resistance, 703
 root-rot, effect of nutrition on, 668
 seed treatment, 405
 survival of *Colletotrichum* on seed of, 24
 Cowpea, 979
 Cox, C. E., (402)
 CROSIER, WILLARD, 92, 162
Crotalaria intermedia, 979
 spectabilis, 340
 Cucumber, 680
 anthracnose, control with Ferimate, 404
 infected by *Pseudomonas lachrymans*, 943
 Pythium on, 782
 Cucumis melo, 653
 var. *inodorus*, 943
 sativus, 321, 653 (See also Cucumber)
Cucurbita maxima, 653
 pepo, 653
Cucurbita occidentalis, 634
Cupressus sempervirens, 775
 Cuproicide (See Fungicides)
 Curly top (See Viruses)
Cuscuta campestris, 396
 spp., parasitic on other dodders, 386
 transmission of virus by, 143, 396
Cymbalaria muralis, 650
Cynoglossum amabile, 654
Cyphomandra betacea, 650
 Cypress, *Coryneum asperulum* on, 775
Cypripedium, 696
Cytospora, 150
 Dahlia pinnata, 647
 DAINES, ROBERT H., 236
 Damping-off, effect of soil moisture, 679
Datura stramonium, 650
Daucus carota, 652
 DAVIDSON, R. S., 237
 DAVIDSON, ROSS W., (777)
 DEAN, LESLIE L., 324
 DECKER, PHARES, (411), 479
 DEMAREE, J. B., 684
 DE ONG, E. R., 469
Dendrobium, 696
 D-D, for control of nematode, 408, 684
 DDT, 405, 407, 681
 control of *Lygus* bug injury to Lima bean, 498
Delphinium cultorum, 654
Diabrotica duodecimpunctata, 514
 vittata, 514
 DIACHUN, STEPHEN, 277
Dianthus barbatus, 647
 chinensis, 647
Diatrype macounii, 408
 Dichloro diphenyl dichloro ethane (See Fungicides; Z-39)
 2, 4-Dichlorophenoxy acetic acid, 505
 2, 3, Dichloro-1, 4-naphthoquinone (See Fungicides; Phygon)
 α -(2,4-Dichlorophenoxy)-*n*-butyric acid, 505
 DICKSON, JAMES G., (226), 397
 DIETZ, S. M., 397
Digitalis purpurea, 650
Dimorphotheca aurantiaca, 647
Diplodia, control of on citrus fruit, 398
 natalensis, 750
 cause of onion rot, 245
 effect of diphenyl vapor on, 888
 theobromae, causing die-back of guayule, 565
 on cotton seed, 35
 zinc, control of on corn seed, 93, 162
 DILLER, J. D., 554
Disodium ethylene bis dithiocarbamate (See Fungicides; Dithane)
Disonycha xanthomelaena, 514
 Dissemination, of air-borne inoculum, 418
 of *Chalaropsis thielavioides*, 286
 of oat mosaic, 359
 Dithane (See Fungicides)
 β , β -Dithiocyano ethyl ether (See Fungicides; B-72)
Dolichos lablab, 649
 DOOLITTLE, S. P., 685
 DORAN, W. L., 679
Dothidella ulci, control of, 688
 resistance of rubber to, 686
Dothiorella, effect of diphenyl vapor on, 888
 Dow fungicides (See Fungicides)
Draculacephala minerva, 118, 401
 DRECHSLER, CHARLES, 213, 781
 DU BEY, H. G., (472)
 DUNN, STEWART, (409)
 du Pont 1452 C (See Fungicides)
 Dusts (See Fungicides)
 effect of diluent on copper availability, 408
 Early blight, resistance of potato, 1011
Echium vulgare, 654
 EDDINS, A. H., 239
 Eggplant, 679, 680
 Elm, chemotherapy, 682
 Dutch Elm disease, 680, 689
Elsinoë piri, distribution of, 458
 EMERSON, R. A., (980)
Emilia flamma, 647
Emmenanthe penduliflora, 648
Empoasca fabae, 514
Endoconidiophora, 284
 fimbriata, effect of diphenyl vapor on, 888
 paradoxa, effect of diphenyl vapor on, 888
 variospora, 284
Endothia parasitica, 554
 ENGLISH, HARLEY, 100
Epidendrum obconicum, 695
Epitrix cucumeris, 514
Erigeron annuus, 647
 speciosus, 647
Erwinia amylovora, 717
 carotovora, in potato tubers, 237
Eryngium aquaticum, viruses of, 402

- Erysiphe cichoracearum*, on zinnia seed, 379
 polygoni, on broad bean, 370
 races of, 373
 host range, 373
viciae pisi f. nov., 378
- ESAU, KATHERINE, (401)
- Ethyl mercuric phosphate (See Fungicides; Semesan Jr.)
- Euchlaena mexicana (See Teosinte)
 inoculation with smut, 411
- Eupatorium lasseauxii, 647
- Euphorbia heterophylla, 653
 marginata, 653
 preslii, 653
- Egopyrum esculentum, 654
- FAIRCHILD, S. J., (481), (897)
- FAWCETT, H. S., 677
- FEASTER, CARL V., (386)
- Fermate (See Fungicides)
- Fern, Bird's-nest, leaf blight, 758
 leaf nematode, 892
- Ferric dimethyldithiocarbamate (See Fungicides; Fermate)
- Festuca (See Grasses)
- FISHER, E. H., 504
- FISHER, HERBERT, (613)
- Fluorine, injury to apricot, 469
- Foliarsphere, 324
- FOLSOM, DONALD, 1016
- Fomes igniarius, 408
- FORSELL, M. J., (458)
- FOSTER, A. A., 680
- FOSTER, R. E., 398, 691
- FRAMPTON, VERNON L., 129
- FRAZIER, NORMAN W., (117), (634)
- FREITAG, J. H., (117), (634)
- Friscanus friscanus, 634
- Fumigation of soil, against nematode, 408, 684
- Fungicides, AAZ (zinc oxide), 728
 Arasan (tetramethyl thiuram disulfide), 289, 403, 687, 728
 castor bean seed treatment, 689
 corn and pea seed treatment, 93
 guayule seed treatment, 1001
 hemp seed treatment, 937
 soybean baldhead control, 169
 soybean seed treatment, 688
 sugar beet seedling blight control, 551
 viability of treated seed, 403, 937
 assay for stability of organic fungicide residues, 679
 B-72 (β , β -dithiocyano ethyl ether), 407
 Bascop, control of tomato fruit rot, 336
 bentonite, 289
 benzyl salicylate-cottonseed oil, control of tobacco blue mold, 648
 bichloride of mercury, sweet-potato dip, 402
 bismuth subsalicylate, control of Alternaria on tomato, 681
 Borax, control of stem-end rot in orange, 750
 sweet-potato dip, 402
 Bordeaux mixture, control of apple fruit spot, 236
 control of Alternaria on tomato, 681
 control of black rot of grapes, 920
 control of cucumber anthracnose, 404
 control of potato late blight, 407
 control of tomato fruit rot, 336
 on potato, 407
 C-119 (copper trichlorophenate), 728
 Ceresan (ethyl mercuric chloride), corn and pea seed treatment, 93
 combinations with growth substances, 687
 copper, action of on seed respiration, 680
 availability as influenced by diluent, 408
 -clay, control of soybean bacterial pustule, 405
 compound A (tetracopper calcium chloride), 407
 control of soybean, bacterial pustule, 405
 on potato, 407
 oxychloride, control of tomato fruit spot, 336
 sulphate, 407
 control of Alternaria on tomato, 681
 control of gardenia leaf spot, 867
 phosphate, 778
 8-quinolinolate, control of apple scab and blotch, 572
 sulphate, in irrigation water as control of tomato fruit rot, 336
 -sulfur, control of soybean bacterial pustule, 405
 Cuprocid (red cuprous oxide), 728
 control of cucumber anthracnose, 404
 guayule seed treatment, 1001
 diphenyl, fungistatic action of, 887
 dips for sweet potato, 402
 Dithane (disodium ethylene bis dithiocarbamate), 407, 681, 685
 D-14, 289
 control of Alternaria on tomato, 681
 control of cucumber anthracnose, 404
 control of tomato wilt, 399
 on potato, 407
 Dow No. 9 (zinc trichlorophenate), 290
 as seed protectant, 406
 cotton seed treatment, 405
 Dow F-48, 407
 du Pont 1452 C (ethyl mercury *p*-toluene sulfonanalide), corn and pea seed treatment, 93
 Fermate (ferric dimethyldithiocarbamate), 687, 778
 castor bean seed treatment, 689
 control of Alternaria on tomato, 681
 control of apple scab and blotch, 572
 control of apple scab and fruit spot, 236
 control of bacterial pustule of soybean, 405
 control of camellia flower blight, 381
 control of cucumber anthracnose, 404
 guayule seed treatment, 1001
 soybean seed treatment, 401
 sweet-potato dip, 402
 vegetable seed treatment, 728
 field evaluation, 686
 glyoxalidine derivatives, 682, 683

- growth regulating effects, 686
 inorganic salts, 728
Isiothan Q15 (lauryl isoquinolinium bromide), 289
 control of apple scab and blotch, 572
 control of apple scab and fruit spot, 236
 on potato, 407
 sweet-potato dip, 402
Isiothan Q32 (cetyl isoquinolinium bromide), control of apple scab and blotch, 572
 leaf structure in relation to spray penetration, 681
 lime, 290
 liquid lime-sulfur, control of apple scab and fruit spot, 236
 Malachite green, 290
Mersolite-19 (phenyl mercuric salicylate), guayule seed treatment, 1001
Merthiolate (sodium ethyl mercurithiosalicylate), control of citrus fruit decay, 398
 Micronized sulfur, control of apple scab and fruit spot, 236
New Improved Ceresan (ethyl mercury phosphate), 405
 control of sugar beet seedling blight, 551
 hemp seed treatment, 937
 soybean seed treatment, 688
 viability of treated seed, 403, 937
New Improved Semesan Jr., castor bean seed treatment, 689
 permeation versus toxicity, 680
 phenothiazine, 729
 phenyl mercuric acetate, 284
 phenyl mercuric chloride, 290
Phygon (2,3, dichloro-1, 4-naphthoquinone), 687
 control of *Alternaria* on tomato, 681
 control of apple scab and fruit spot, 236
 control of turf copper spot, 403
 corn and pea seed treatment, 93
 guayule seed treatment, 1001
 on potato, 407
 sweet-potato dip, 402
Puratized (phenyl mercuri triethanol ammonium lactate), control of *Alternaria* on tomato, 681
 117, control of turf copper spot, 403
N5-E, control of apple scab and blotch, 572
 control of black scale of lily, 392
 control of citrus fruit decay, 398
 on potato, 407
 sweet-potato dip, 402
N5-X, control of apple scab and fruit spot, 236
 control of black scale of lily, 392
Puraturf, control of copper spot of turf, 403
o-quinone dioxime, 680
Semesan (hydroxy mercurichlorophenol), 728
 castor bean seed treatment, 689
 guayule seed treatment, 1001
 viability of treated forage crop seed, 403
Semesan Bel (hydroxy mercuri nitrophenol + hydroxy mercuri chlorophenol), sweet-potato dip, 402
Semesan Jr. (ethyl mercuric phosphate), corn and pea seed treatment, 93
 soybean baldhead control, 169
 sodium dimethyl dithiocarbamate, 290
 sodium orthophenylphenate, control of citrus fruit decay, 398
 sodium sulfate, 680
Spergon (tetrachloro parabenzonquinone), 403, 687, 728
 castor bean seed treatment, 689
 control of *Alternaria* on tomato, 681
 corn and pea seed treatment, 93
 growth regulating effects, 686
 guayule seed treatment, 1001
 hemp seed treatment, 937
 soybean baldhead control, 169
 soybean seed treatment, 401
 sweet potato dip, 402
 viability of treated seed, 403, 937
 Yellow calla seed treatment, 701
Spergonex (*o*-benzoquinone dionium peroxide), guayule seed treatment, 1001
 spraying technique, 680
 sulfur, causing reduction in yield of pear, 777
 control of bacterial pustule of soybean, 405
 control of bean rust, 689
 control of sweet-potato soil rot, 869
Tenn. 34, control of bacterial pustule of soybean, 405
Tersan (tetramethyl thiuram disulfide), 728
 castor bean seed treatment, 689
 control of apple fruit spot, 236
 sweet potato dip, 402
Tetrachlororesorcinol, 728
Thiosan (See Tersan)
Thiourea, control of citrus fruit decay, 398
Tribasic copper sulfate, 407
 control of *Alternaria* on tomato, 681
 control of cucumber anthracnose, 404
 control of tomato fruit rot, 336
U.S.R. No. 604 (See Phygon)
Yellow eucroide, 407
 control of tomato fruit rot, 336
 on potato, 407
 viability of treated forage crop seed, 403
Z-39 (dichloro diphenyl dichloro ethane), 407
Zerlate (zinc dimethyldithiocarbamate), 407
 control of *Alternaria* on tomato, 681
 control of bacterial pustule of soybean, 405
 control of copper spot on turf, 403
 on potato, 407
 sweet-potato dip, 402
 Zinc ethylene bisdithiocarbamate, 685
 Zinc sulfate, 290

- Fusarium*, 152, 252, 400, 679
 moniliforme, cause of mile root rot, 410
 on cotton seed, 29
 on corn seed, control with fungicides, 93, 162
 on cotton seed, 35
 on pea seed, control with fungicides, 93
 on sugar beet seed, 394
 oxysporum, f. asparagi, 397
 f. cubense, 983
 f. lycopersici, first symptom on tomato, 691
 f. tuberosi, 902
 seirpi, effect of diphenyl vapor on, 888
 spp., on cereal seed, 399
 variofectum, 703
 wilt of tomato, effect of soil moisture and temperature, 218
- Gaillardia aristata*, 647
Galinsoga parviflora, 647
Gamolepis tagetes, 647
GARBER, R. J., (403)
Gardenia, bacterial leaf spot of, 865
Garlic, virus of, 292
GARNER, JAMES M., (557)
Gas, illuminating, causing abscission, 505
GATTANI, MOHAN LAL, 398
GÜDMANN, ERNST, 624
Genetics of microorganisms, Phoma lingam, variation in, 408
 Gibberella zeae, relation to adaptation, 396
 Ustilago zeae, diploid lines, 398
 Venturia inaequalis, 403
 Xanthomonas phaseoli, variation in, 589, 613
- Geranium*, 687
 carolinianum, 648
GERHARDT, FISK, (100)
Geum canadense, 654
 chiloense, 654
Gibberella zeae, 400
 adaptation in relation to genetic variation, 396
GIDDINGS, N. J., 38, 58
Gilia capitata, 650
 liniflora, 650
GILLY, C. L., (410)
Glococercospora sorghi, control of on turf grass, 403
Gloeosporium perennans, effect of diphenyl vapor on, 888
 necator, 1043
 venetum, 1043
- Glomerella*, on apple, 528
 on lupine, 528
 on *Chamaecrista*, 528
 cingulata, 394
 causing anthracnose of *Lespedeza*, 524
 effect of diphenyl vapor on, 888
 effect of growth substances on, 394
 races of, 532
Glycine max, 649
Glyoxalidine derivatives, as fungicides, 682, 683
GODFREY, G. H., 398
GOLDSMITH, G. W., (667)
- Gomphrena haageana*, 646
GORDON, W. L., 399
Gossypium spp. (See Cotton)
 hirsutum, 654
GOTT, CORA LEE TERRY, 667
GOTTLIEB, DAVID, 399, 557
GRAHAM, T. W., (684)
Grape, muscadine, black rot on, 905
 Pierce's disease virus of, 117, 401, 634
Graphium ulmi, 682
Grasses, Blue-, stripe smut of, 57, 404
 Brome-, *Xanthomonas* on, 446
 Festuca elatior, crown rust on, 404
 var. *arundinacea*, crown rust on, 404
 Johnson, sooty stripe of, 200
 Sudan, sooty stripe of, 200
 viability of treated seed, 403
 Turf, copper spot control, 403
GREANEY, F. J., 252
Green dwarf, of potato, 671
GRIES, GEORGE A., 399, (680)
Growth regulating effects of fungicides, 686
Growth substances, effect on *Glomerella cingulata*, 394
 in combination with fungicides, 687
GRÜENHAGEN, R. H., 399
Guayule, *Diplodia* die-back of, 565
 seed treatment, 999
Guignardia bidwellii, 913
 f. *muscadinii* f. nov., on grape, 913
Gypsophila elegans, 647
- HANSEN, H. N.*, (381)
HANSING, E. D., 400, 433
HANSON, E. W., 400
HARRAR, J. G., 400, (479)
HARRY, J. B., (683)
HEDGES, FLORENCE, 589, 613, 678
HEIBERG, BARBARA C., (245), 887
Helianthus annuus, 647
 tuberosus, 647
Helicrysum bracteatum, 647
Heliopsis scabra, 648
Heliotropium corymbosum, 654
Helipterum humboldtianum, 648
Helminthosporium, on oats, 406, 407
 carposaprum, sp. nov., 991
 gramineum, method of inoculating barley, 689
 sporulation in culture, 1049
 sativum, 252
 effect of crop rotation on, 410
 on barley, 397
 turcicum, on corn, 412, 660
Helochara delta, 119, 401
Hemp, seed treatment, 937
HENDERSON, R. G., 400
HENRY, B. W., (1056)
Heterodera marioni, 340, 408, 684
 rostochiensis, 180, 688
HEUBERGER, J. W., (399), 685, 686
Hevea, control of leaf blight, 688
 resistance to leaf blight, 686
HEWITT, WM. B., 117, (401)
HEYNE, E. G., (400), (433)
Hibiscus esculentus, 654
 manihot, 654

- HILDEBRAND, A. A., 401
Ippeastrum puniceum, 653
 HODGSON, ROLAND, 401
Ipoleus sudanensis, 653
 HOLLAND, A. H., (493)
 HOLMES, FRANCIS O., 643
 HOOKER, W. J., 389
 HOPKINS, E. F., (750)
 HOPPERSTEAD, S. L., (326)
Hordeum vulgare, 653 (See also Barley)
 Hormones, reduction of bud, blossom, and pod drop in string bean, 504
 HORSFALL, JAMES G., (399), 680
Hosta japonica, chondriosomal changes in, 472
 HOUSTON, BRYON R., (117), 401, 1049
 HUMPHREY, HARRY B., 87
 HUNGERFORD, C. W., (324)
Hunneemannia fumariacifolia, 649
 HUTCHINS, LEE M., (409)
Hydroxy mercuri chlorophenol (See Fungicides; Semesan)
Hypericum boreale, 653

Iberis, *Pythium* on, 782
 gibbularia, 648
 sempervirens, 648
 umbellata, 648
Impatiens balsamina, 646
 holstii, 646
Incarvillea variabilis, 654
Indole-3-acetic acid, 505
(Indole-3)- γ butyric acid, 505
 Insecticides, control of *Lygus* bug on bean, 498
 DDT, 405, 407, 681
 Pyrethrum, 505
 Rotenone, 519
 Insects, abscission induced by, control, 514
 injury to Lima bean, 493
 spittle insect injury, burn blight of pine, 399
 transmission of disease, 418
 Insects as vectors, alfalfa virus, by aphids, 142
 big vein of lettuce, by aphids, 264
 onion yellow dwarf, by aphids, 293
 Pierce's disease of grape and alfalfa, 118, 401, 634
 witches' broom of alfalfa, by leafhoppers, 772
Ipomoea batatas (See Sweet potato), 344, 648, 979
 nil, 648
 setosa, 648
 tricolor, 648
 , Isothan (See Fungicides)

 JACOBSON, H. G. M., (399)
 JEFFERS, W. F., 402, 686
 JENKINS, ANNA E., 458, 1043
 JOHNSON, E. M., 112, 142
 JOHNSON, FOLKE, 384
 JOHNSON, JAMES, 402
 Johnson grass, sooty stripe of, 200
 JONES, F. R., 1057
 JONES, R. C., (409), (681)

Kalanchoe diagremontiana, 654
 KEIL, HARRY L., 403
 KEINHOLZ, J. R., 778
 KEITT, G. W., 1, 403, (406)
 KENT, G. C., (389)
 KO, SIANG YIN, 226
 KOCH, L. W., (401)
Kochia scoparia, 647
 KOELLER, BENJAMIN, 403, 937
 KREITLOW, K. W., 403, 404
 KREUTZER, W. A., 329
 KUNTZ, J. E., 404

 LACKEY, C. F., 388, 462
Lactuca sativa (See Lettuce)
 scariola, 979
Lamium amplexicaule, 649
 LANGFORD, M. H., 686
Lantana camara, 345, 652
 LARSON, R. H., 404
 Late blight, fungicides for, 407
 on tomato, 389
 spread of, 423
 technique for epidemiology study, 322
 water-congestion and infection, 402
Lauryl isoquinolinium bromide (See Fungicides; Isothan Q15)
Lavatera trimestris, 654
 LEACH, J. G., 57, 404, 876
 LEACH, L. D., (549)
 Lead arsenate, 573
 LEBEAU, F. J., 393, 404
 LEBEN, CURT C., (402), 405
 LECHLER, E. L., 1011
 LEFEBVRE, C. L., (190), (688)
 LEHMAN, S. G., 405
Lens esculenta, 649
Leptosyne maritima, 648
Lespedeza, 979
 anthracnose, 524
 stipulacea, susceptibility to anthracnose, 522
 striata, susceptibility to anthracnose, 532
 Lettuce, 679
 big vein of, 264
 seed treatment, 729
 susceptible to *Phytomonas aptata*, 552
 LEUCKEL, R. W., (688)
Levulinic acid, 505
Lilium longiflorum, var. *eximium*, 391
 philippinense, 653
 Lily bulbs, treatment with fungicides and growth substances, 686
 Lima bean (See Bean; Lima)
 Lime (See Fungicides)
Limonium bonduelli, 654
 sinuatum, 654
Linaria maroccana, 650
 vulgaris, 650
Linum flavum, 653
 grandiflorum, 654
 perenne, 654
Lobelia spp., hosts for tobacco viruses, 649
Lobularia maritima, 648
 LOCKE, WARREN F., (380)
 LOEGERING, W. Q., (400)
 LOMBARD, FRANCES F., 777
 LOTCKS, K. W., 750

- LOWTHER, C. V., (57)
Luffa acutangula, 653
Lunaria annua, 648
 Lupine, factors affecting seedling survival, 479
Lupinus angustifolius, 479
 hartwegii, 649
 LUTTBELL, E. S., 905
Lycium coeli-rosa, 647
Lycium chinense, 650
Lycopersicon (See also Tomato)
 chilense, 650
 esculentum, 651, 693
 birsutum, 651
 resistance to mosaic, 685
 peruvianum, 651
 pimpinellifolium, 651, 693
Lycopus rubellus, 649
Lygus elisus, causing Lima bean injury, 502
 hesperus, causing Lima bean injury, 502
 oblongatus, 514
- MACHNER, J. H., 686
Macrophoma zeae, 204
Macrophomina phaseoli, effect of diphenyl vapor on, 888
 pycnidial strain of, 978
Macrosiphum solanifolii, 264
Macrosporium, 679
 effect of diphenyl vapor on, 888
 on sugar beet, 394
 sarcinaeforme, 680
Macrosteles divisus, 514
 Mahonia, as alternate host of stem rust, 400
 Malachite green, 290, 396
Malope trifida, 654
Malva rotundifolia, 654
 Maple, Eutypella canker of, 408
 MANNS, T. F., 686, (686)
 Manuscript preparation, 953
 Mariana plum, 284
Marmor cepae (See Viruses; onion yellow dwarf)
 erodens (See Viruses; tobacco etch)
 phaseoli (See Viruses; bean mosaic)
 tabaci (See Viruses; tobacco mosaic)
 terrestre, sp. nov., 366
 var. *typicum*, 367
 var. *oculatum*, 367
Marrubium vulgare, 649
Mathiola bicornis, 648
 incana, 648
 McCALLAN, S. E. A., 405, (682)
 McLELLAN, W. D., 687
 McCORLOCK, L. P., 988
 McCRIBB, W. A., 687
 McDONNELL, A. D., (680)
 McKENZIE, M. A., 680
 McKINNEY, H. H., 359
 Media, Quirk's, for bacteria, 592
Medicago sativa, 649 (See also Alfalfa)
 MEEHAN, FRANCES, 406, (407)
Melanconium, effect of diphenyl vapor on, 888
Melanospora, effect of diphenyl vapor on, 888
 MELCHERS, L. E., (410)
- MELHUS, I. E., (410), (411)
Melissa officinalis, 649
 Melon, honeydew, bacterial spot of, 943
Mentha spicata, 649
 MENZIES, J. D., 762
 MEREDITH, CLIFFORD H., 406, 983
 Merthiolate (See Fungicides)
Mesembryanthemum crystallinum, 646
 linearis, 646
 MEULI, LLOYD J., 406
 MILBRATH, J. A., 671
 MILLS, W. D., 353
 Milo disease, 410
Mimulus moschatus, 650
 tigrinus, 650
 MINZ, GERSHON, 383
Mirabilis jalapa, 654
Mollugo verticillata, 646
Momordica charantia, 653
Monilinia fructicola, effect of diphenyl vapor on, 888
 laxa, effect of diphenyl vapor on, 888
 MOORE, J. DUAIN, 406
 MOROFSEY, W. F., (407)
 MORRIS, H. E., (394)
 MUNCIE, J. H., 407
 MURPHY, H. C., (406), 407
 Muskmelon, seed treatment, 730
Mycosphaerella brassicaicola, life cycle of, 481
 MYERS, W. M., (404)
Myosotis scorpioides, 654
 sylvatica, 654
Myzus convolvuli, 264
 persicae, 264, 293
- NAGEL, C. M., 407
 α -Naphthalene acetic acid, 505
 β -Naphthoxy acetic acid, 505
 α -Naphthyl acetamide, 505
Narcissus, virus of, 292
 Nematode, D-D for control of, 408, 684
 golden, 180, 686, 688
 as a quarantine problem, 687
 on bird's-nest fern, 892
 Panagrolaimus subelongatus, 213
 root knot of tobacco, 340, 684
 root rot of tobacco, 684
Nemesia strumosa, 650
Nemophila insignis, 648
 maculata, 648
Neokolla circellata, 119, 401
 spp., virus vectors, 634
Nepeta cataria, 649
 hederacea, 649
 nussini, 649
 NEWHALL, A. G., 896, (982)
 New Improved Ceresan (See Fungicides)
 New Improved Semesan (See Fungicides)
Nicotiana physalodes, 651
Nicotiana glauca, 387
 glutinosa, 645
 curly-top virus in, 464
 megalosiphon, 340
 plumbaginifolia, 340
 spp., hosts for tobacco viruses, 651, 652
 tabacum, 340
 curly-top virus in, 464

- Nierembergia hippomanica*, 652
 NIKITIN, A. A., 408
Nolana lanceolata, 649
 NUSBAUM, C. J., 18, 164
 Nutrition, of cotton in relation to wilt, 703
 magnesium, in relation to cotton root rot, 668
 manganese as a cause of apple measles, 395
 of bean and susceptibility to *Xanthomonas phaseoli*, 613
 of Japanese chestnut in relation to blight, 554
 of onion in relation to yellow-dwarf virus, 297
 of potato in relation to leaf roll, 409
 of tomato in relation to defoliation by *Alternaria*, 681
 Onk wilt, 397
 Oats, mosaic of, 359
 new *Helminthosporium* disease of, 406
 reaction to loose smut, 433
 reaction to stem and crown rust, 226
 resistance to *Helminthosporium*, 407
 seed microflora of, 399
 O'BRIEN, MURIEL, (410)
Oenothera lamarekiana, 654
 OLIVE, LINDSAY S., 190
 Onion, 679
 Diplodia rot of, 245
 reaction to onion-yellow-dwarf virus, 294
Oospora lactis parasitica, effect of diphenyl vapor on, 888
Ophiotrichum, on cotton seed, 29
 Orange, stem-end rot, 750
 stubborn disease, 675
 Orchid, bacterial disease, 695
 ORTON, C. R., 241
 OSWALD, J. W., (1049)
Oxalis, 395
 corniculata, 654
 stricta, 654
Pagaronia spp., virus vectors, 634
 PALMITER, D. H., 681
Papaver nudicaule, 649
 orientale, 649
Paratiroza cockerelli, 899
 PARRIS, G. K., 408
Parthenium argentatum (See Guayule)
Pastinaca sativa, 652
 PATRICK, STEWART, (163)
 Pea, 680
 Pythium on, 781
 seed treatment, 92, 729
 water-congestion and infection, 402
 Peach, blotch, 273
 mosaic, 396
 transmission of wart virus, 675
 Pear, bacterial blight, 717
 geographical distribution of *Elsinoë piri* on, 458
 reduction in yield by sulfur spray, 777
Pelargonium hortorum, 648
 Armillaria root rot of, 302
 odoratissimum, 648
Pellicularia microsclerotia, effect of diphenyl vapor on, 888
Penicillium, 679
 control of on citrus fruit, 398
 control of on corn seed, 93
 digitatum, effect of diphenyl vapor on, 888
 expansum, effect of diphenyl vapor on, 888
 effect of ultraviolet radiation on spores of, 102
 italicum, effect of diphenyl vapor on, 888
 notatum, 290
 on cotton seed, 34
 oxalicum, 403
 Penicillin, chemotherapy with, 717
Penstemon grandiflorus, 650
 Pepper, 679, 680
 tobacco-etch virus on, 685
Peronospora manchurica, on alfalfa and soybean, 1057
 PERSON, L. H., 869
Pestalotia, effect of diphenyl vapor on, 888
 PETERSON, W. H., (401)
Petroselinum hortense, 652
Petunia hybrida, 652
Phacelia spp., hosts for tobacco viruses, 649
Phalacrognathus, 695
Phaseolus (See also Bean)
 spp., hosts for tobacco viruses, 649
 Phenothiazine (See Fungicides)
 Phenoxy acetamide, 505
 acetic acid, 505
Phenyl mercuri triethanol ammonium lactate (See Fungicides; Puratized)
Phlox drummondii, 650
Pholiotia squarrosoides, 408
Phoma betae, effect of diphenyl vapor on, 888
 destructive, effect of diphenyl vapor on, 888
 lingam, variation in, 408
 on sugar beet, 394
 pomi, control of on apple, 236
Phomopsis, 152
 citri, 750
 effect of diphenyl vapor on, 888
 vexans, effect of diphenyl vapor on, 888
Phosphorus and wheat stem rust, 557
Phragmidium rubi-idaei, races of, 383
 Phygon (See Fungicides)
Phyllacora graminis, on corn, 412
Phyllosticta strominella, effect of diphenyl vapor on, 888
Phymatotrichum omnivorum, 668
Physalis alkekengi, 652
 angulata, 652
 peruviana, 645, 652
 subglabrata, 652
Physalospora zeae, cause of corn ear rot, 201
 Physiologic specialization, *Cercospora oryzae*, 395, 950
 Corticium solani, 401
 Erysiphe polygoni, 373
 Glomerella cingulata, 532
 Phragmidium rubi-idaei, 383
 tobacco-etch virus, 685
 Ustilago avenae, 400, 434
 Physiology of microorganisms, *Corticium solani*, 401

- Glomerella cingulata*, 694
Pseudomonas lachrymans, 945
Streptomyces griseus, 412
Venturia inaequalis, 405
Phytolacca decandra, 650
Phytomonas aptata, seed transmission of, 549
 asplenii n. sp., 758, 892
 cattleyae, 695
 juglandis, 717
 maculifolium-gardeniae n. sp., 865
 tumefaciens, 400
Phytophthora capsici, on tomato, 329
 citrophthora, effect of diphenyl vapor on, 888
 infestans, 322, 407 (See also Late blight)
 on tomato, 389
Pine, burn-blight and spittle insect injury, 399
Piricularia oryzae, 1056
Pisum sativum, 649, 781 (See also Pea)
Plantago lanceolata, 650
 major, 650
 rugelii, 650
Plastids, seasonal changes of, in *Hosta*, 472
Platyloideus acutus, 771
Plectospora myriandra, 845
Pleospora lycopersici, effect of diphenyl vapor on, 888
Poa pratensis (See Bluegrass)
Polanisia trachysperma, 647
POLLACK, F. C., (988)
Polyamia inimicus, 514
Polygonum hydropiper, 654
POMERLEAU, RENE, 408
Poplar, canker of, 148
Populus (See Poplar)
Poria punctata, 408
PORTE, W. S., (685)
PORTER, R. H., 168
Portulaca oleracea, 650
Potato, control of nematodes with D-D, 684
 early blight control with zinc ethylene bisdithiocarbamate, 685
 Erwinia carotovora in tubers, 237
 golden nematode of, 686, 688
 green dwarf, 671
 hair sprout in relation to psyllids, 899
 internal necrosis of tubers, 480
 late blight, dissemination of, 423
 fungicides for, 407
 net necrosis, 686, 1016
 nutrition in relation to leaf roll, 409
 resistance to early blight, 1011
 scab, 388, 411
 and calcium-potassium ratio, 399
 in relation to soil pH, 682
 Sclerotium rot of seed pieces, 239
 sprays and dusts, 407
 stem-end browning, 925, 1016
Potentilla arguta, 654
 mousseliensis, 654
POUND, GLENN S., 408, 1035
POWELL, DWIGHT, 572
Pratylenchus, 684
Prennity, 627, 892
PRESLEY, JOHN T., 565
PRICE, W. C., 157
Primula malacoides, 650
 obconica, 650
Proboscidea louisiana, 649
Prunella vulgaris, 649
Prunus armeniaca, *Armillaria* root rot of, 302
 persica, *Armillaria* root rot of, 302
 spp., hosts for cherry tatter-leaf virus, 74
 hosts for peach-blotch virus, 275
PRYOR, DEAN E., 170, 264
Pseudomonas lachrymans, causing spot of honeydew melon, 943
 medicaginis var. *phaseolicola*, overwintering of, 677
 syringae, 677
Psyllids, causing internal necrosis of potato tubers, 480
 in relation to hair sprout of potato, 899
Puccinia (See also Rust)
 coronata, 226, 404, 418
 glumarum, 410
 graminis, 400
 avenae, 226, 400
 tritici, 381, 400, 557
 rubigo-vera, 410
 sorghii, 395
Pullularia, effect of ultraviolet radiation on spores of, 102
Puritized (See Fungicides)
Pyrax ABB, 505
Pyrethrum, 407
Pythium, 403
 anandrum, 839
 arrhenomanes, 410
 effect of crop rotation on, 410
 control of damping-off caused by, 679
 debaryanum, 169
 effect of diphenyl vapor on, 888
 effect of crop rotation on, 410
 development and morphology of species of, 781
 graminicola, 169
 oligandrum, development of, 781
 on sugar beet, 394
 periplocum, development of, 803
 salpingophorum, development of, 805
 ultimum, seed treatment of peas against, 93
 undulatum, 846
 vexans, 819
QUANJER, H. M., 892
Quercus spp., hosts for *Chalara quercina*, 397
o-Quinone dioxime (See Fungicides)
Radish, infection by *Actinomyces scabies*, 388
RAMSEY, GLEN B., 245, (887)
Ramulispora sorghi comb. nov., causing sooty stripe of sorghum, 198
RAND, F. V., (1)
RANDS, R. D., 688
Ranunculus asiaticus, 654
Raphanus raphanistrum, 648
 sativus, 648
Raspberry, mild streak virus of, 402
 yellow rust of, 383

- RAY, W. W., 409
 REEVES, E. L., 409, (409)
Reseda odorata, 654
 Resistance, of cabbage to mosaic, 408
 celery to *Cercospora*, 980
 corn to *Helminthosporium*, 412, 660
 cotton to bacterial blight, 409
 to wilt, 703
 grape to black rot, 910
 Hevea to leaf blight, 686, 688
 Lima bean to mosaic, 170
 oats, 688
 to *Helminthosporium*, 407
 to rust, 226
 onion to *Diplodia* rot, 248
 potato to early blight, 1011
 rice to *Cercospora*, 950
 tobacco to mosaic, 412, 685
 to root knot and nematode root rot, 684
 tomato to fruit rot, 338
 types of, in plants, 624
 Respiration, as affected by fungicides, 680
 Rhizoctonia, on lupine, 479
 on sugar beet, 394
 solani, effect of diphenyl vapor on, 888
 effect of temperature on pathogenicity, 638
 seed treatment of pea against, 93
 on soybean, 411
 Rhizopus, effect of diphenyl vapor on, 888
 effect of ultraviolet radiation on spores of, 102
 nigricans, control of on corn seed, 93, 162
 on cotton seed, 29
 Rice, 395
 reaction to *Cercospora oryzae*, 950
 RICHARDS, B. L., 409, (409)
 RICHARDS, C. AUDREY, (399)
 RICHARDS, M. C., 409, 681
Ricinus communis, 653 (See also Castor bean)
 RIKER, A. J., (399), (401), (504), 953
 ROBERTS, E. A., (681)
 ROBERTS, JOHN W., 175
 RODRIGUEZ V., JOSE, 410
 Root diseases, technique for study of, 667
 Root knot, of tobacco, 684
 Root rot, of asparagus, 399
 of cereals and grasses, effect of crop rotation on, 410
 of milo, 410
 of tobacco, 400
 of wheat, 252
 Rosa, *Armillaria* root rot of, 302
 odorata, 654
 Rose, failure of bud and graft unions caused by *Chalaropsis thielavioides*, 281
 ROSS, A. FRANK, 925
 Rot, control of in oranges, 750
 of deciduous trees, 408
 of muscadine grapes, 905
 of orchids, 695
 of sweet potato, control, 869
 of tomato fruit, 988
 pole-rot of tobacco, 679
 Rubber (See Hevea)
Rubus, 1043
Rudbeckia hirta, 648
 RUDOLPH, B. A., 717
 RUE, JOHN L., (396)
Ruga verrucosa (See Viruses; curly top)
Rumex crispus, 654
 obtusifolius, 654
 RUPERT, JOSEPH A., (726)
 Rust, bean, control with sulfur, 689
 corn, 395
 crown, reactions of *Festuca* to, 404
 oats crown, dissemination of, 418
 reactions of oat hybrids to, 226
 oats stem, reactions of oat hybrids to, 226
 pine blister, dissemination of, 422
 raspberry, 383
 stem, in Mexico, 400
 sunflower, 402
 teosinte, 395
 wheat leaf, dissemination of, 424
 in Mexico, 410
 wheat stem, and phosphorus distribution, 557
 in relation to yellow berry, 381
 wheat stripe, in Mexico, 410
 RYALL, A. L., (398)
 RYAN, MARY A., (57), (404), (876)
Saintpaulia ionantha, 648
Salpiglossis sinuata, 652
Salvia spp., hosts for tobacco viruses, 649
Sanvitalia procumbens, 648
 Scab, on potato, influence of pH, 682
Scabiosa atropurpurea, 653
 caucasica, 653
 SCHALLER, C. W., (534)
Schinus molle, *Armillaria* root rot of, 302
Schizanthus pinnatus, 652
Sclerospora graminicola, on corn, 410
Sclerotinia camelliae, 380
 fructicola, effect of ultraviolet radiation on spores of, 102
 intermedia, effect of diphenyl vapor on, 888
 minor, effect of diphenyl vapor on, 888
 sclerotiorum, causing pole-rot of tobacco, 679
 effect of diphenyl vapor on, 888
Sclerotium rolfsii, effect of diphenyl vapor on, 888
 rot of potato seed pieces, 239
Secale cereale, 653
 Seed injury, by Lygus bug, 493
 in relation to infection by *Xanthomonas translucens* var. *cerealis*, 450
 Seed transmission of bacterial blight of sugar beet, 549
 of milo disease, 410
 Seed treatment, 406
 castor bean, 689
 cotton, 405
 effect on respiration, 670
 guayule, 999
 hemp, 937
 peas and sweet corn, 92
 soybean, 169, 401, 688
 sweet potato, 402
 vegetables, 727
 viability of stored seed, 403, 937

- SEMIENIUK, G., 410, (411)
 Senecian (See Fungicides)
Senecio cineraria, 345
Septoria citri, effect of diphenyl vapor on, 888
 lycopersici, effect of diphenyl vapor on, 888
 musiva, canker on hybrid poplars, 148
 Shallot, virus of, 292
 SHANDS, H. L., 534
 SHAY, J. R., (403)
 SHEAR, C. L., (1043)
 SHERWIN, HELEN S., (190), 688
Silene anglica, 647
 pendula, 647
Sinningia speciosa, 648
 SLAGG, C. M., 410
 SMITH, FLOYD F., (292)
 SLEETH, B., 909
 SMITH, M. A., 943
 SMITH, OLIVER F., 638
 Smut, barley loose, reaction of barley varieties to, 534
 bluegrass stripe, 59
 cytology of, in culture, 404, 876
 hunt, effect on other diseases of wheat, 400
 of wheat in Mexico, 410
 corn, method of inoculation, 411
 variation in, 398
 oats loose, races of, 400, 433
 reaction of oat varieties to, 433
 of teosinte, 395
 wheat flag, in Mexico, 479
 wheat loose, dissemination of, 418
 yield reduction by, 1040
 Snapdragon, 687
 SNYDER, WILLIAM C., 481, 484, (493), 897
 Sodium ethyl mercurithiosalicylate (See Fungicides; Merthiolate)
 Sodium metaborate, as control of citrus fruit decay, 398
 Sodium orthophenylphenate (See Fungicides)
 Soil, fumigants, D-D mixture, 408
 transmission of big vein of lettuce, 264
Solanum melongena var. *esculentum*, infected by *Phytophthora aptata*, 552
 spp., hosts for tobacco viruses, 652
 tuberosum, (See Potato)
Solidago rugosa, 648
 Sorghum, 979
 halapense, 192
 sooty stripe of, 190
 vulgare, 192
 Sori sporium, on teosinte, 395
 SOUTHWICK, M. D., (681)
 Soybean, brown stem rot, 394
 bud blight caused by virus, 319
 control of bacterial pustule by dusts, 405
 downy mildew, 1057
 induced baldhead of, 168
 relation of stomatal behavior to infection by *Xanthomonas phaseoli* var. *sojense*, 385
 Rhizoctonia solani, 411
 seed treatment, 401, 688
 Spergon (See Fungicides)
Sphaceloma necator comb. nov., 1047
 Spinach, 680
 seed treatment, 729
Spinacia oleracea, 647 (See also Spinach)
 SPRAGUE, RODERICK, 410
 STAKMAN, E. C., (400), 411, (479)
 STANTON, T. R., (400), (433), 688
 STEINER, G., 688
 STEINMETZ, F. II., 682
Stellaria media, 647
 Stem-end browning, of potato, 925
 STEVENS, KAY, 411
 STEVENSON, E. C., 689
 STEVENSON, FRANK V., (411)
 STEVENSON, FREDERICK J., (87)
 STEVENSON, JOHN A., 487
 STODDARD, E. M., 682
 Strawberry, virus of, 684
Streptomyces griseus, nutrition of, 412
 STRONG, M. C., 218
 STUART, NEIL W., (279), (687)
 Sudan grass (See also Grasses; Sudan)
 sooty stripe of, 200
 Sugar beet, 394
 Pythium on, 782
 curly-top virus concentration in, 38
 mass action as a factor in infection by curly-top virus, 53
 seed transmission of bacterial blight, 549
 Sulfur (See Fungicides)
 Sunflower, 979
 Sweet potato, 979
 growth regulating effects of fungicides on, 686
 internal brown spot, 164
 internal cork, 18
 Pythium on, 781
 soil rot, control with sulfur, 869
Symplocarpus foetidus, 653
Syncephalastrum, effect of diphenyl vapor on, 888
Systema taeniata, 514
 Tagetes spp., hosts for tobacco viruses, 648
 TAKAHASHI, WILLIAM L., (129)
 TAPKE, V. F., 167, 689
Taraxacum kok-saghyz, 346
 officinale, 648
 TAYLOR, CARLTON F., 411, 726
 Technique, Aerosol treatments for tobacco
 blue mold control, 684
 aids for study of potato-late-blight epidemiology, 322
 artificial induction of abscission of bean leaves, 504
 assay of stability of organic fungicide residues, 679
 determination of virus in meristems, 462
 diphenyl, for control of contaminants, 887
 electrophoretic studies of plant viruses, 129
 for field evaluation of fungicides, 686
 for inoculation, *Helminthosporium* of barley, 689
 loose smut of barley, 536
 loose smut of oats, 433
 Ustilago striiformis of bluegrass, 65
 Ustilago zeae of Maydeae, 411
 wetting and adhesive agents, 1056

- for study of root diseases, 667
 radio-active elements in the study of
 physiology of disease, 557
 seed and seedling infection with *Xantho-*
monas translucens var. *cerealis*, 446
 serological method for distinguishing
 southern bean mosaic virus, 157
 single ascospore isolations from apo-
 thecia, 167
 spraying, interactions of factors, 680
 Temperature, effect on baldhead of soy-
 bean, 169
 brown stem rot of soybean, 394
 die-back of guayule, 565
Fusarium wilt of tomato, 218
 golden nematode of potato, 187
 growth of *Glomerella cingulata*, 531
Helminthosporium foot rot of barley,
 397
 infection by *Xanthomonas translucens*
 var. *cerealis*, 448
 necrotic ring spot viruses, 406
 out mosaic, 359
 pathogenicity of *Chalaropsis thielavioides*
 on rose, 286
 potato net necrosis and stem-end brown-
 ing, 1016
 powdery mildew of broad bean, 372
Rhizoctonia solani, 638
 seed treatment of hemp, 937
 seed treatment of soybean, 688
 sporulation of *Helminthosporium*, 1049
 tomato fruit rot, 330
 wilt and root rot of asparagus, 397
 yellows virus of sour cherry, 353, 406
 Temperature of soil, effect on big vein of
 lettuce, 269
 effect on incidence of root rot of wheat,
 254
 relation to *Armillaria* root rot, 302
 relation to root canker of alfalfa, 638
 Tenn. 34 (See Fungicides)
 Teosinte, diseases of, 395, 411
 Tersan (See Fungicides)
 TERVET, IAN W., 411
 Tetrachlororesorcinol (See Fungicides)
Tetragonia expansa, 646
 Tetramethyl thiuam disulfide (See Fungi-
 cides; Arasan)
Thelesperma hybridum, 618
 THIEGS, BERNARD J., (406)
Thielaviopsis basicola, 284, 401
 Thiosan (See Fungicides; Tersan)
 Thiourea (See Fungicides; Thiourea)
 THOMAS, H. EARL, (231), 381, (481),
 (695), (897)
 THOMAS, W. D., 324
 THORNBERRY, H. II., 412
Thunbergia alata, 653
 THURSTON, H. W., 683
 TIFFANY, LEWIS HANFORD, 327
 TIFFANY, LOIS, (411)
Tilletia (See also Smut; bunt)
 caries, 400, 410
 foetida, 400, 410
 levis (See *Tilletia foetida*)
 tritici (See *Tilletia caries*)
 Tobacco, control of blue mold with Aerosol
 treatments, 684
 curly-top virus in, 462
 etch (See Viruses)
 mosaic (See Viruses)
 pole-rot of, 679
 resistance, to mosaic, 412, 685
 to root knot and nematode root rot,
 684
 to root rot, 400
 water-congestion and infection, 402
 Tomato, 349, 679, 979
 bushy stunt virus, 157
 cuticle cracking of fruit, 413
 fruit rot of, 329, 988
 fungicides in control of *Alternaria*, 681
Fusarium wilt, 218, 399, 691
 late blight, 389
 nutrition in relation to defoliation by
Alternaria, 681
 Pythium on, 782
 seed treatment, 729
 water-congestion and infection, 402
 wilt, caused by bacterial toxins, 401
 TOMPKINS, C. M., 699, (758), (892)
Torenia fournieri, 650
 TORRIE, JAMES H., (226), (1057)
 TOWNSEND, G. R., 980
Trachymene caerulea, 652
 Transmission, of alfalfa virus, 142
 of alfalfa witches' broom, 767
 of bacterial blight of sugar beet, 549
 of big vein of lettuce through soil, 264
 of green dwarf of potato, 672
 of mild disease, 410
 of onion yellow-dwarf virus, 293
 of Pierce's disease of grape, 118, 634
 of plant diseases by insects, 418
 of strawberry virus, 684
 Tribasic copper sulfate (See Fungicides)
 Trichlorophenol, zinc salt of (See Fungi-
 cides; Dow 9)
Trichoderma viride, effect of diphenyl
 vapor on, 888
Trifolium incarnatum, 649
 pratense, 649
 repens, 649
 virus of, 144
 2,3,5-Triiodobenzoic acid, 505
Tripsacum dactyloides, inoculation with
 smut, 411
Triticum aestivum, 653 (See Wheat)
Tropaeolum majus, 654
 TSIANG, C. T., (411)
Tubercularia, causing cankers on *Ulmus*
pumila, 395
 Tulip spot, dissemination of, 424
 TULLIS, E. C., (395), (950)
 Turf grass (See Grasses; turf)
 TURNER, NEELY, (680)
Ulmus pumila, 284
 canker of, 395
Urocystis tritici, in Mexico, 479
Ustilago avenae, 433
 races of, 400
 dieteliana, 411
 kellermanii, on teosinte, 395

- kolleri, 433
 nuda, 534
 scitaminea, 411
 striiformis (striaeformis), chlamydospore
 germination and culture, 59
 f. poae-pratensis, cytology of, 404, 876
 method of inoculating bluegrass with,
 65
 zeae, adaptation to arsenic, 411
 method of inoculation, 411
 variation of, 398
 ULLSTRUP, ARNOLD J., 201, 412

 VALLEAU, W. D., (112), (277), 412
 Variation, in *Gibberella zeae*, 396
 in *Phoma lingam*, 408
 in *Ustilago zeae*, 398
 in *Xanthomonas phaseoli*, 589, 613
 Venturia inaequalis, physiology of, 405
 pyrina, 777
 Verbascum phoeniceum, 650
 Verbena spp., hosts for tobacco viruses,
 652
 Veronica spp., hosts for tobacco viruses,
 650
 Vicia faba, 649 (See also Bean; broad)
 infected by *Phytomonas aptata*, 552
 sativa, 649
 Vigna sinensis, 649
 Vinca rosea, 653
 Viola arvensis, 654
 Viruses, alfalfa witches' broom, 762
 bean mosaic, 324
 association with bacteria, 589
 bean virus No. 2, 394
 bean virus No. 4, electrophoretic studies
 of, 137
 big vein, transmission of in lettuce, 264
 blueberry stunt, 684
 cabbage mosaic, resistance to, 408
 control of, 1035
 cauliflower virus No. 1, 409
 cherry wilt and decline, 409
 cucumber mosaic No. 1, electrophoretic
 studies of, 137
 cucumber virus No. 1, 404
 curly-top, concentration in sugar beet,
 38
 in meristematic tissue, 462
 mass action as factor in infection
 of sugar beet, 53
 electrophoretic studies of, 129
 garlic virus, reaction of onion varieties
 to, 292
 inhibitors in spinach extract, 404
 lima-bean mosaic, 170
 mild streak of raspberry, 402
 narcissus virus, reaction of onion va-
 rieties to, 292
 necrotic ring spot, effect of temperature
 on, 406
 oats mosaic, 359
 of alfalfa and white clover, 142
 of *Eryngium*, 402
 of sweet cherry, 409
 onion-yellow-dwarf virus, effect of nitro-
 gen on susceptibility of onion to,
 297
 reaction of onion varieties to, 292
 peach blotch, transmission of, 273
 peach mosaic, forms of, 396
 peach wart, transmission of, 675
 Pierce's disease of grape, 401, 634
 insects as vectors, 634
 on alfalfa and grape, 401
 potato green dwarf, 671
 potato leaf roll, effect of nutrition on,
 409
 potato ring spot, 157, 404
 potato veinbanding, 157
 potato X, electrophoretic studies of, 136
 potato Y, electrophoretic studies of, 137
 shallot virus, reaction of onion varieties
 to, 292
 sour cherry yellows, 353, 406
 relation of temperature to, 406
 southern bean mosaic, antigenicity of,
 157
 stone fruit-virus cultures, ring-spot con-
 taminant, 396
 strawberry, 684
 stubborn disease of citrus, 675
 tatter leaf of sweet cherry, 73
 tobacco etch, 157
 host range of, 643
 on peppers, 685
 tobacco mosaic, 157, 396
 electrophoretic studies of, 134
 field strains of, 112
 host range of, 643
 tobacco varieties resistant to, 412
 wild tomato resistant to, 685
 tobacco necrosis, 157
 tobacco ring spot, causing bud blight of
 soybean, 319
 tobacco virus No. 1, 404
 tomato bushy stunt, 157
 turnip virus No. 1, 404, 409
 Vitis spp., inoculated with *Guignardia*, 919

 WALKER, E. A., 689
 WALKER, J. C., (398), (404), 415
 WALLIN, J. R., (410), 412, 446
 Walnut, bacterial blight of, 717
 black, 284
 Persian, 284
 WATERMAN, ALMA M., 148
 Watermelon, 408
 Pythium on, 782
 WEIMER, J. L., 524
 WEINDLING, RICHARD, 413
 WELLMAN, R. H., 682
 WEST, ERDMAN, (239)
 WESTER, ROBERT E., (170)
 Wheat, bacteria in association with roots
 of, 277
 bunt of, in Mexico, 410
 effect of bunt on other diseases, 400,
 effect of fusarial head blight on bunt,
 400
 factors influencing root rot of, 252
 flag smut in Mexico, 479
 foot rot, 400

- Pythium* on, 782
 seed microflora of, 399
 seedling blight, 400
 seedling infection with *Xanthomonas translucens* var. *cerealis*, 446
 stem rust and phosphorus distribution, 557
 stem rust in relation to yellow berry, 381
 water-congestion and infection, 402
 yield reduction by loose smut, 1040
 WHITTAKER, C. W., (554)
 WIAIT, JAMES S., (245)
 WILLISON, R. S., 73, 273
 WILSON, C. T., (411)
 WILSON, E. E., 418
 Wilt, of asparagus, 397
 of cherry, 409
 of chestnut, 682
 of oak, 397
 of tomato, 218, 399, 691
 Witches' broom of alfalfa, 762
 of corn, 410
 Woods, M. W., (402), 472
Xanthomonas juglandis, 717
 malvacearum, 413
 phaseoli, dissociation of, 589, 613
 overwintering of, 677
 var. *sojense*, 277, 385
 control of by dusts, 405
 translucens var. *cerealis*, races of, 446
 seedling infection with, 446
 vesicatoria, on wheat roots, 277
 Yellow berry of wheat, 381
 Yellow calla, 699
 Yellow euprocide (See Fungicides)
 YOUNG, P. A., 391, 413
 YU, T. F., 370
 Z-39 (See Fungicides)
Zaluzianskya villosa, 650
Zantedeschia elliottiana, 699
 ZAUMEYER, W. J., 689
Zea mays, 653 (See Corn)
 Zerlate (See Fungicides)
Zinc ethylene bisdithiocarbamate (See Fungicides)
Zinc trichlorophenate (See Fungicides; Dow)
Zinnia elegans, 648
 haageana, 648

ERRATA, VOLUME 35

Page 1008, line 1, *read* *Pseudomonas* *for* *Pseudomonas*

Page 1009, line 39, *read* inoculation techniques *for* infection techniques

ERRATA, VOLUME 36

Page 14, line 15, *read* Ann. Rept. 6: 84. 1892 (1893). *for* Ann. Rept. 5: 84. 1892-93

Page 22, table 1, footnote c, *read* Moderate, 5 to 10 spots per root. footnote c, *read* Weighted average of scale, none to 4, inclusive

Page 37, item 2 in literature cited, *read* Karon *for* Katon

Page 38, line 14 and Page 58, line 20, *read* verrucosans *for* verrucosa

Page 75, line 38, *read* season *for* sesaon

Page 92, line 30, *read* reduced the number of growths of *Diplodia* *for* reduced the *Diplodia*

Page 511, figure legend and Page 512, line 15, *read* abscisable *for* abscissable

Page 549, line 20, *read* and in severe form near Klamath Falls, Oregon, and Alturas, California, at elevations above 4,000 feet *for* at elevations above 4,000 feet

Page 591, line 17, *read* special media *for* media

Page 593, line 5, *read* extract as for yeast medium I *for* extract and yeast solution as for medium I

Page 611, line 16, *read* noninoculated *for* inoculated

Page 648, table 1, line 2, *read* *Helipterum humboldtianum* *for* *Helipterum humboltianum*

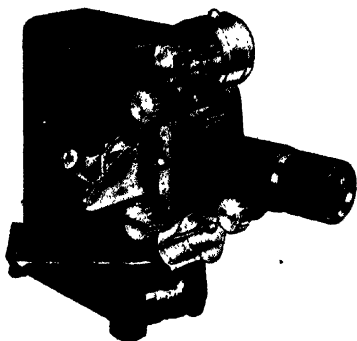
Page 680, line 22, *read* G. A. Gries *for* A. Gries

Page 754, paragraph 5, *read* The low humidity room was held at $75 \pm 0^\circ$ F. and 56-57 per cent relative humidity, and the high humidity room was held at $79 \pm 3^\circ$ F. and a relative humidity of 90-94 per cent

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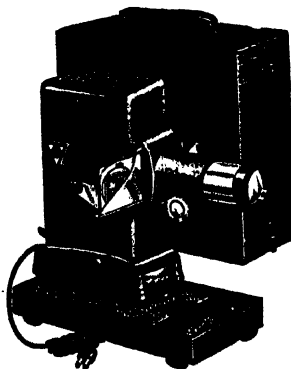
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